Synthesis of Unnatural Analogues of Pancratistatin and Narciclasine

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ABSTRACT

Described herein is the chemoenzymatic synthesis of several different types of unnatural analogues of Amaryllidaceae constituents. Development and refinement of existing and design and execution of new approaches towards the synthesis of C-1 analogues of pancratistatin and A-ring heterocyclic analogues of narciclasine are discussed. Evaluation of the new analogues as cancer growth inhibitory agents is also described.

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LIST OF ABBREVIATIONS

2,2-DMP	2,2-dimethoxypropane
AcOH	Acetic acid
AD-mix	Asymmetric dihydroxylation mixture
AIBN	2,2′-Azobis(isobutyronitrile)
Bn	Benzyl
BOP	(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
BTMSA	*bis*-Trimethylsilyl acetylene
CDI	1,1'-Carbonyldiimidazole
Cp	Cyclopentadienyl
Cp*	Pentamethylcyclopentadienyl
$CpCo(CO)_2$	Cyclopentadienylcobalt dicarbonyl
CyJohnphos	2-(Dicyclohexylphosphino)biphenyl
DBU	1,8-Diazabicycloundec-7-ene
DCC	*N,N′*-Dicyclohexylcarbodiimide
DCN	1,4-dicyanonaphthalene
DEAD	Diethyl azodicarboxylate
DHP	2,3-dihydropyran
DIBAL	Diisobutylaluminium hydride
DIPEA	*N,N*-Diisopropylethylamine
DMIPS	Dimethylisopropylsilyl
DMP	Dess-Martin periodinane
DPPA	Diphenylphosphoryl azide
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EE	Ethoxyethyl
Ee	Enantiomeric excess
Er	Enatiomeric ratio
EVE	Ethoxyvinyl ether
HBTU	O-Benzotriazole-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate
HOBt	Hydroxybenzotriazole
LHMDS	Lithium bis(trimethylsilyl)amide
LDA	Lithium diisopropylamide
LTMP	Lithium 2,2,6,6-tetramethylpiperidide
*m*CPBA	*m*-Chloroperoxybenzoic acid
Me	Methyl

MOM	Methoxymethyl
Ms	Methanesulfonate
NaHMDS	Sodium bis(trimethylsilyl)amide
*n*Bu	*n*-Butyl
NMO	*N*-Methylmorpholine *N*-oxide
*n*Pr	*n*-Propyl
*o*DCB	*o*-Dichlorobenzene
P(o-Tol)3	*tris*(*ortho*-tolyl) phosphine
Ph	Phenyl
PhMe	Toluene
PivCl	Pivaloyl chloride
PMB	*p*-Methoxylbenzyl
pMBDMA	*p*-Methoxybenzaldehyde dimethylacetal
PMP	*p*-Methoxyphenyl
PPTS	Pyridinium *p*-toluene sulfonate
SEM	2-(Trimethylsilyl)ethoxymethyl
SMEAH	Sodium bis(2-methoxyethoxy)aluminum hydride
TBAF	Tetra-*n*-butylammonium fluoride
TBAT	Tetra-*n*-butylammonium triphenyldifluorosilicate
TCDI	1,1'-Thiocarbonyldiimidazole
TDO	Toluene dioxygenase enzyme
TES	Triethysilyl
Tf	Trifluoromethylsulfonate
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic acid anhydride
TIPS	Triisopropylsilyl
TMEDA	*N,N,N′,N′*-Tetramethylethylenediamine
Ts	*p*-Toluenesulfonyl
UHP	Urea-hydrogen peroxide complex

1. Introduction

Since the discovery and isolation of narciclasine (**1**) in 1968 by Ceriotti[1] and pancratistatin (**2**), Figure 1, by Pettit[2] in 1984 the scientific community has been drawn towards these molecules because of their potent antineoplastic activity. The highly selective cytotoxicity towards malignant cells ensured the interest of the community in developing potential drugs based on their molecular pattern of Amaryllidaceae constituents. However, their preclinical development is significantly hampered by the relatively low abundance of **1** and **2** in natural sources as well as their poor water solubility.

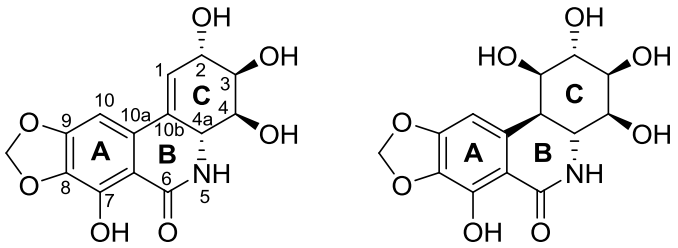


Figure 1. Structure of narciclasine and pancratistatin.

Synthetic organic chemistry is an indispensable tool which can address both of these issues by developing efficient ways to synthesize of the different analogues of **1** and **2** which may also help to establish the biological mechanism of action of these fascinating molecules. The main goal of this thesis will be to devise new ways and refine existing approaches towards synthesis of C-1 analogues of pancratistatin such as **5 a-c** and A-ring heterocyclic analogues of narciclasine of type **6** and **7**, Figure 2. Biological

evaluation of these synthetic compounds will be performed in order to provide us with new insight towards mechanism of action and help to refine minimal pharmacophore requirements.

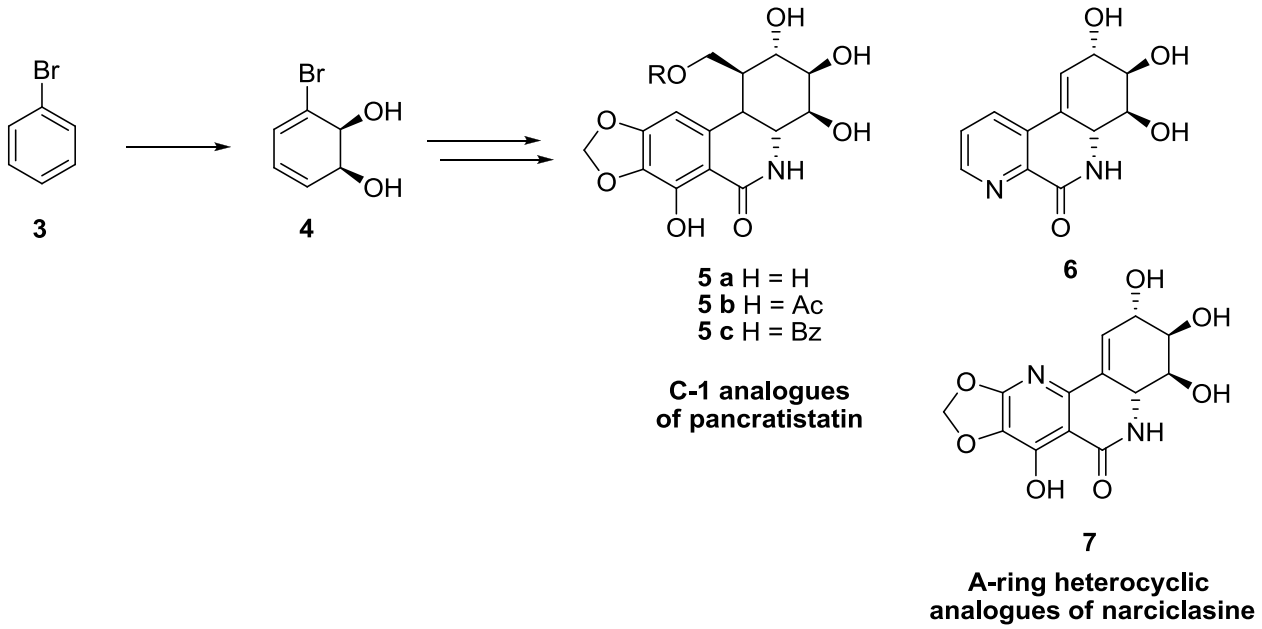


Figure 2. General strategy for synthesis of analogues of pancratistatin and narciclasine.

The unifying theme of all these approaches will be the use of a highly versatile chiral building block – *cis*-cyclohexadiene diol **4**. The utilisation of such a chiral building block for the synthesis of complex organic targets has been a common theme in the Hudlický group for many years. This compound is obtained by biooxidation of bromobenzene **3** by the whole cell fermentation with strain of *E. Coli* JM109 (pDTG601A). Diol **4** will be used in the synthesis of unnatural derivatives **5 a-c**, **6**, **7**. When completed these compounds will be evaluated by screening against cancer cell lines.

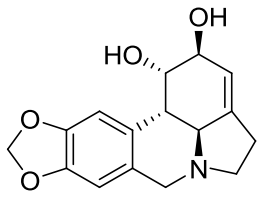
2. Historical

2.1. Amaryllidaceae alkaloids

2.1.1. Discovery and biosynthesis

Amaryllidaceae is a large family of flowering perennial plants. There have been used for a long time in folk medicine for treatment of various ailments because of the high amounts of various bioactive alkaloids. In the ancient Greek and Roman medicine the plants of the *Narcissus* genus and their essences were used for the treatment of tumours and cancer–like diseases.[3]

In 1877 the first alkaloid from this family was isolated from *N. pseudonarcicuss* and named lycorine (**8)**, Figure 3.[4] It is one of the most abundant alkaloids in the family and possesses some antitumoral activity. More than 100 members of this family of alkaloids were subsequently isolated and despite their shared isoquinoline structures they belong to structurally different groups. Here our attention will be focused on the group of these alkaloids which share the isocarbostyril structural motif, namely (**9**).



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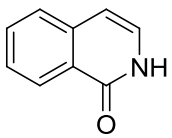

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Figure 3. Structure of lycorine and isocarbostyril.

Compounds with the highest anticancer activity were isolated and characterised in the late 20th century. All of these congeners are highly oxygenated compounds and share the same structural isocarbostyril pattern (**9**). The first of this class of naturals products, narciclasine (**1**), was isolated in 1968,[1] closely followed by isolation of 7-deoxynarcilasine or lycoricidine, (**10**).[5] Two decades later pancratistatin (**2**) was isolated by Pettit *et al.* in 1984,[2] and 7-deoxypancratistatin (**11**) was isolated by Ghosal in 1989.[6] *trans*-Dihydronarciclasine (**12**) was produced first semi-synthetically in 1975[7] from narciclasine and only in 1990 was isolated from a natural source by Pettit.[8] The latest of the active compounds was 7-deoxy-*trans*-dihydronarciclasine (**13**) isolated in 1993 by the same group,[9] Figure 4.

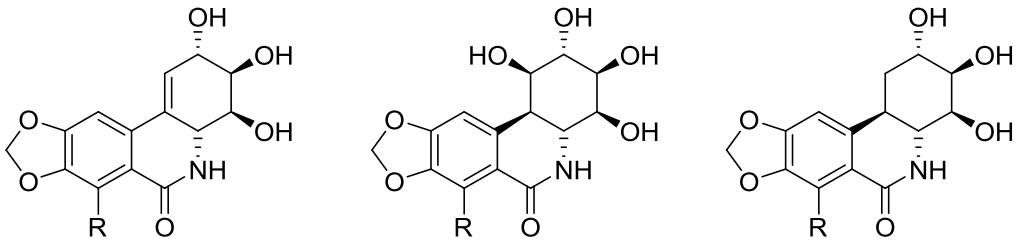


Figure 4. Three major isocarbostyril congeners of Amaryllidaceae family and their respective 7-deoxy versions.

While the exact biosynthetic pathway towards these compounds is unknown, it is reasonable to assume that they all share common biosynthetic origin. Two major starting materials for biogenesis of all alkaloids of Amaryllidaceae family are phenylalanine (**14**) and tyrosine (**17**). They undergo transformation into protocatechuic aldehyde (**15**) and tyramine (**18**) respectively. Coupling of those two compounds followed by reduction and

methylation leads to the central intermediate in the biosynthesis - *O*-methylnorbelladine (**19**), Figure 5.[10]

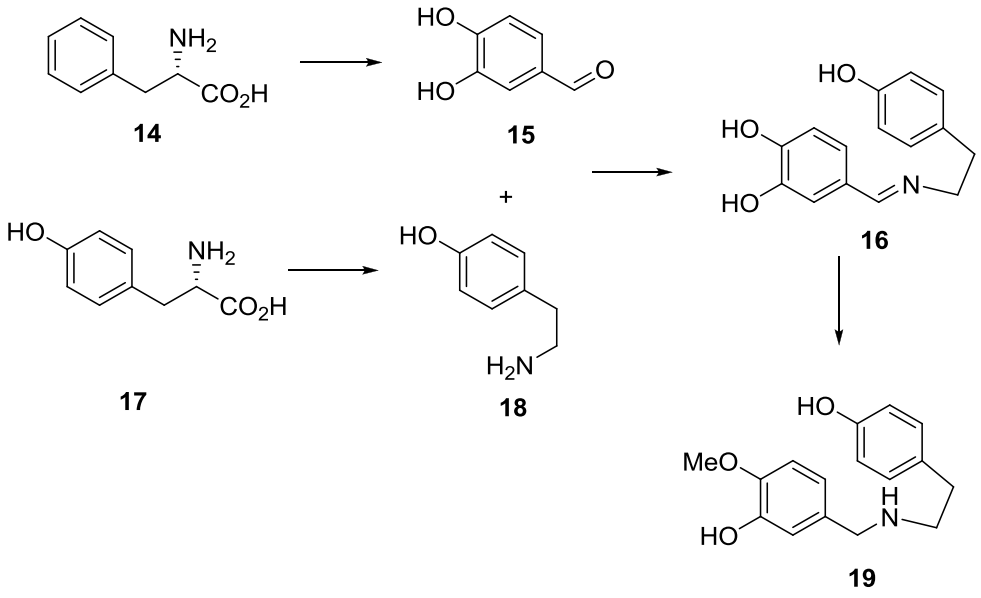


Figure 5. Biosynthetic pathway to a common intermediate *O*-methylnorbelladine (**19**).

The general pathway to phenanthridine type alkaloids proceeds *via* oxidative cyclization of **19**. Different types of regioselective oxidative cyclization lead to a wide variety of products. The main question is whether a *para-para* or *para-ortho* regioselective coupling is involved in the particular case of narciclasine (**1**). Studies of the biosynthetic pathway were performed with labeled compounds to show that narciclasine is most likely produced *via* a *para-para* phenol oxidative process.[10] Oxidation of *O*-methylnorbelladine (**19**) followed by Michael addition yields noroxomaritidine (**20**). Oxidation of this intermediate is thought to produce narciclasine in series of oxidation steps,[10] Figure 6.

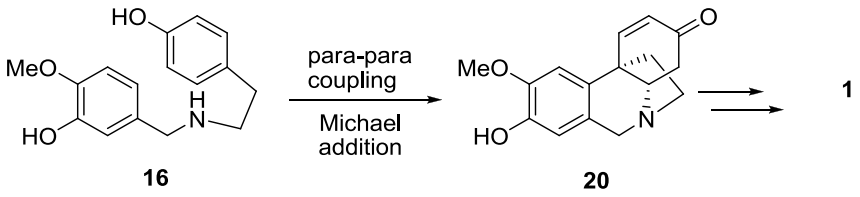


Figure 6. Transformation of *O*-methylnorbelladine to narciclasine.

The exact biosynthetic route has not yet been established for other congeners of narciclasine such as **2, 10-13**. But as all of these compounds share a similar structure, it is reasonable to assume that they all share the same biosynthetic pathway.

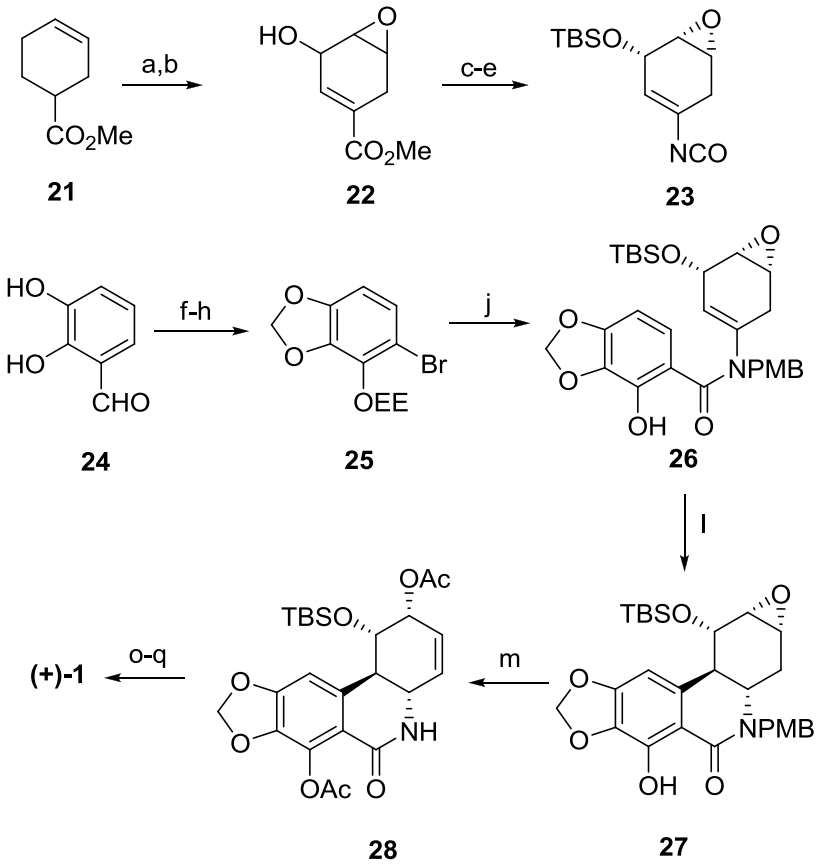
2.1.2. Selected total syntheses.

2.1.2.1. Total syntheses of narciclasine.

The goal of this section is not to present all existing syntheses of narciclasine and pancratistatin-type molecules, which have been the topic of a number of reviews.[4, 10-22] First racemic and first enantioselective synthesis will be presented in detail. A few other selected syntheses, especially those published after 2008, will be shown in an abbreviated form.

Despite being the earliest of all isocarbostyril congeners discovered and displaying the most potent anticancer properties, narciclasine (**1**) has received the least attention from a synthetic standpoint. Indeed, only four total syntheses of this compound are known to date. The first synthesis of narciclasine (**1**) was reported by Rigby and Matteo[23] in 1997. Their synthetic strategy involved the transformation of the commercially available methyl

ester of the cyclohexene carboxylate acid **21** to epoxyalcohol **22**, which was converted to a chiral isocyananate **23** by lipase resolution, Scheme 1. Reaction of the lithium derivative of **25** with this isocyanate led to amide **26**, which upon photocyclization conditions furnished the full framework of **1**, Scheme 1. A similar strategy was utilised by this group three years later in the synthesis of pancratistatin.[24]



Reaction conditions: (a) NBS, AIBN, *n*Bu_3SnH, 75%; (b) (i) O_2, rose bengal, hν; (ii) $RuCl_2(PPh_3)_2$, CH_2Cl_2; (iii) NaOMe, MeOH, 42%; (c) (i) *n*PrCOCl, Et_3N; (ii) cholesterol esterase, 40%; (d) (i) TBSCl, imidazole, 52%; (ii) LiOH, MeOH, H_2O, 42%; (e) (i) DPPA, Et_3N, benzene; (ii) PhMe, reflux; (f) CH_2Br_2, K_2CO_3, DMF, 84%; (g) (i) *m*CPBA, (ii) KOH, EtOH, 63%; (h) CF_3CO_2Ag, Br_2; (ii) EVE, PPTS, 85%; (j) (i) *n*BuLi, THF; (ii) **23**, 52%; (k) (i) PMBBr, NaH; (ii) PPTS, MeOH, 76%; (l) hν, benzene, 46%; (m) (i) $(PhSe)_2$, $NaBH_4$, (ii) H_2O_2; (iii) AcCl, NaH, 48%; (o) (i) OsO_4, NMO, *t*BuOH; (ii) 2,2-DMP, TsOH, 76%; (p) (i) TBAF, THF; (ii) Burgess reagent, benzene, 64%; (q) (i) K_2CO_3, MeOH; (ii) *n*BuLi, THF, O_2; (iii) TsOH, 37%.

Scheme 1. Rigby`s first enantioselective synthesis of narciclasine.

In the second reported synthesis of **1** Hudlický[25, 26] used chiral cyclohexadiene diol **29** as a C-ring fragment for synthesis of narciclasine. This diene was submitted to a hetero-Diels-Alder reaction to provide the key bicyclic intermediate **30**. Suzuki coupling of this compound with arylboronic acid **31** and reduction led to enone **32**, which, after installation of the correct stereochemistry in position 2 and cyclization was followed by deprotection, furnishing narciclasine (**1**) in twelve steps.

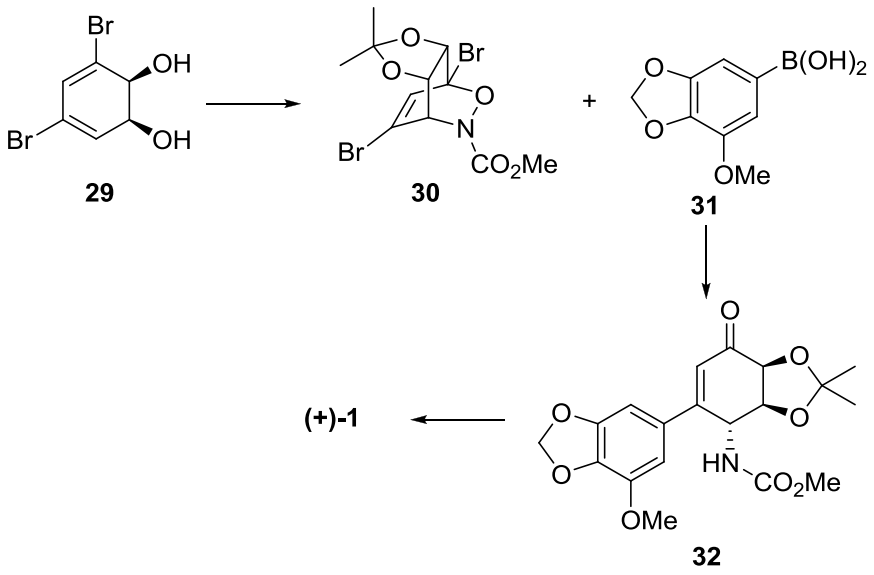


Figure 7. Hudlický`s synthesis of narciclasine.

The third synthesis of narciclasine was reported by Keck *et al*.[27] In 1999 their group published a general approach towards both natural narciclasine and *ent*-lycoricidine. The pivotal point of their synthesis is an intramolecular 6-*exo* radical cyclization between a radical generated from alkyne and oxime **35**, Figure 8. Chirality in this synthesis was achieved by using a chiral pool starting material, namely D-gulonolactone **33**, as a precursor to alkyne **34**.

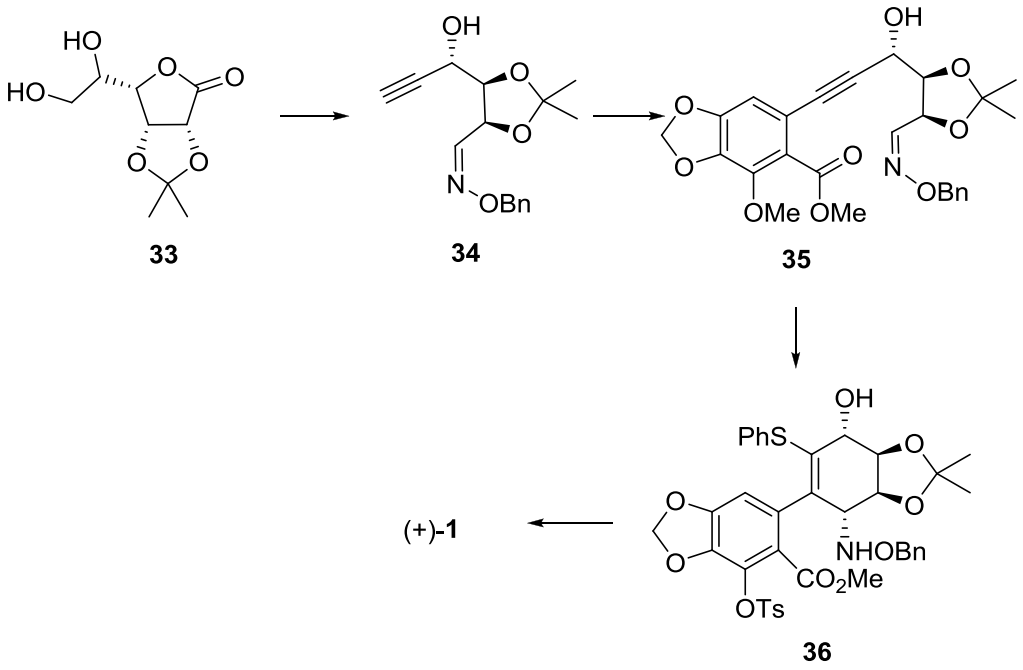
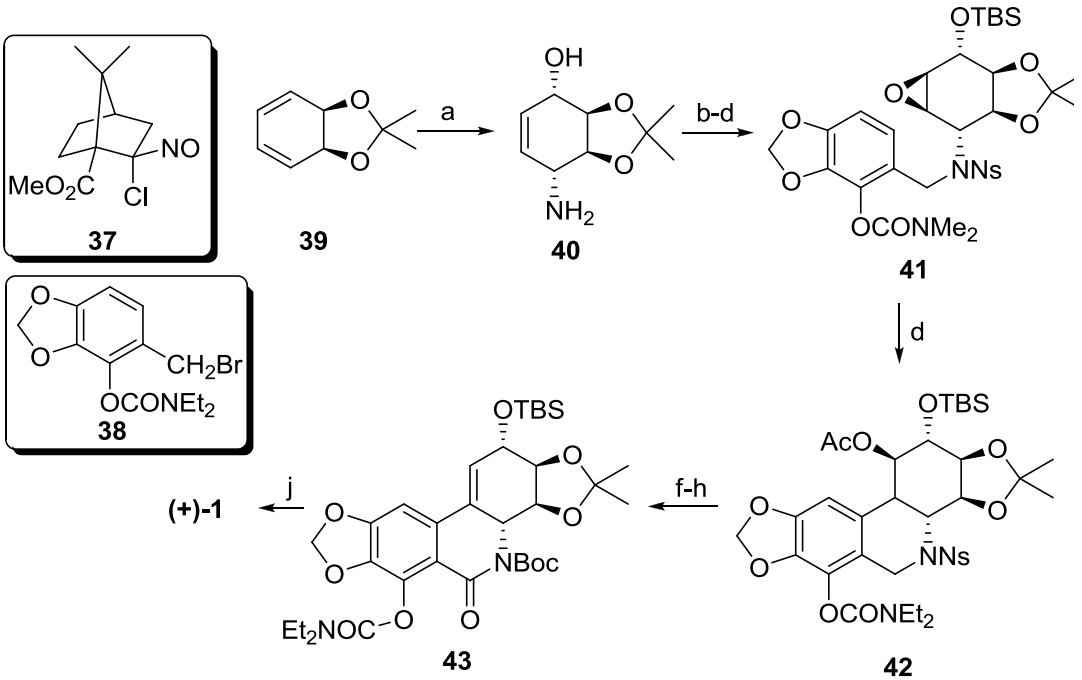


Figure 8. Keck`s synthesis of narciclasine.

The most recent synthesis of **1** was reported in 2002 by Elango and Yan.[28] Their approach to the construction of narciclasine was similar to that previously reported by Hudlický for the synthesis of 10-epi-7-deoxypancratistatin[26, 29] and consists of intramolecular opening of epoxide **41** to form phenanthridine **42**, Scheme 2.The starting material used in their synthesis was the achiral cyclohexadiene diol **39** produced by the whole-cell oxidation of benzene. In order to transform it to the chiral conduramine **40** an enantioselective hetero-Diels-Alder reaction with chiral nitroso compound **37** was performed. In a few steps, that included bromohydrine formation and alkylation, the key epoxide **41** was prepared. Intramolecular Lewis acid-catalyzed epoxide opening led to the tricyclic product **42**, which upon benzylic oxidation and elimination provided protected narciclasine **42**. Finally, deprotection gave enantiomerically pure narciclasine (**1**) in ten steps.



Reaction conditions: (a) (i) **37**, CH_2Cl_2; (ii) Al-Hg, MeCN, 85%; (b) (i) NsCl, DBU, Et_3N, MeCN; (ii) TBSCl, DBU, rt, 78%; (c) NBS, acetone, H_2O, 98%; (d) **38**, K_2CO_3, MeCN, 88%; (e) (i) $SnCl_4$, CH_2Cl_2; (ii) Ac_2O, K_2CO_3, 98%; (f) thioglicolic acid, LiOH, DMF, 78%; (g) (i) Boc_2O, MeCN; (ii) $RuCl_3$, $NaIO_4$, H_2O, 67%; (h) DBU, benzene, 96%; (j) (i) HCOOH, THF; (ii) LAH, THF, 65%.

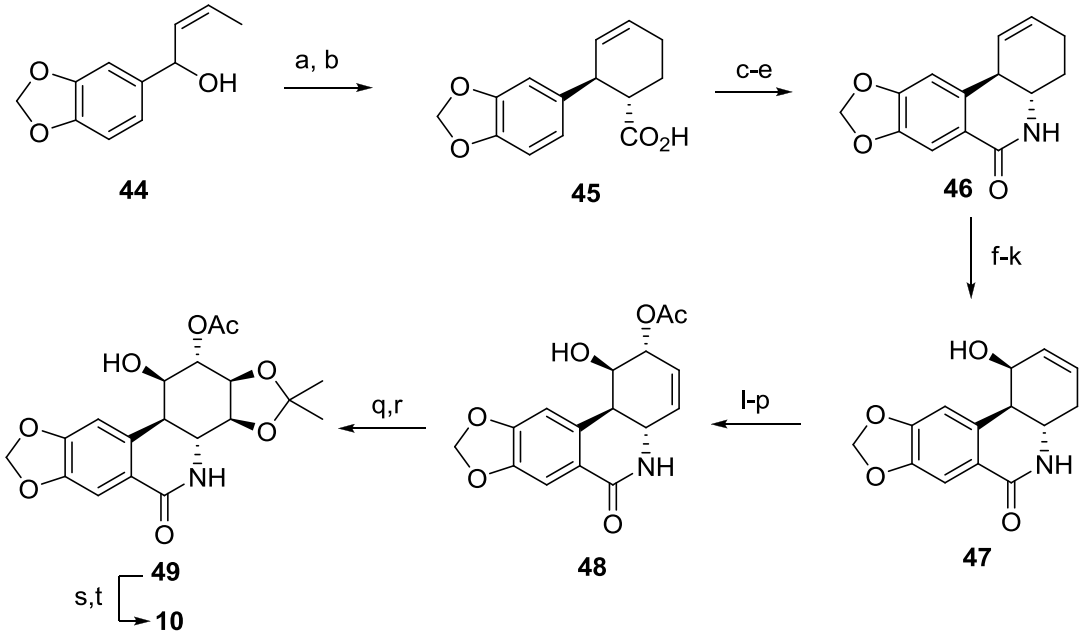
Scheme 2. Yan`s synthesis of narciclasine.

2.1.2.2. Total syntheses of lycoricidine.

Lycoricidine (**10**) has attracted much more attention as a synthetic target and constitutes the earliest example of an isocarbostyril Amaryllidaceae alkaloid made by synthesis. The first racemic synthesis was performed by Ohta and Kimoto.[30] Incidentally, on the way to this compound they had also prepared the protected version (**49**) of another related natural product, then as yet undiscovered 7-deoxypancratistatin (**11**).

The synthesis began with the allylic alcohol **44**, a precursor for a diene whose Diels-Alder reaction with ethyl acrylate provided, after hydrolysis, acid **45**, Scheme 3. After Curtius rearrangement and electrophilic cyclization tetracycle **46** was further

functionalised to establish the four hydroxyl stereocenters of 7-deoxypancratistatin. Finally, elimination of the unprotected C-1 alcohol moiety in **49** led to lycoricidine (**10**).

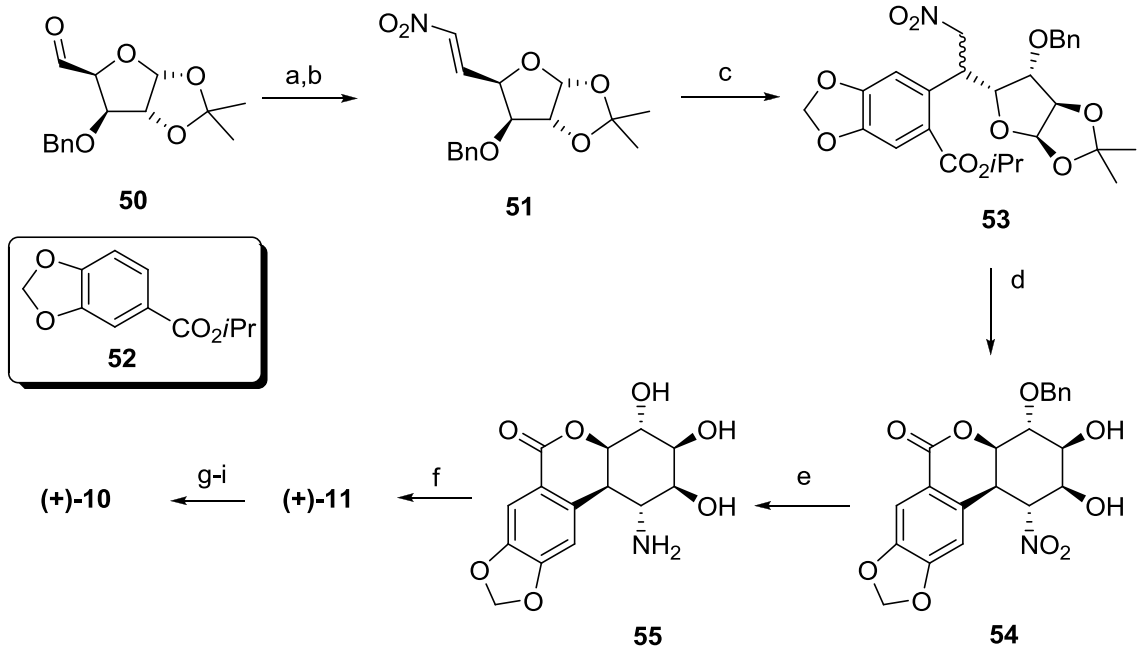


Reaction conditions: (a) ethyl acrylate,TsOH, 56%; (b) NaOEt, EtOH, (ii) H_2O, 74%; (c) $ClCO_2Et$, Et_3N, acetone, H_2O; 42%; (d) (i) NaN_3, H_2O; (ii) toluene, reflux 40%; (e) BF_3*Et_2O, 89% for 3 steps; (f) Ac_2O, pyridine, 83%; (g) KOH, EtOH, 68%; (h) NBS, THF, 96%; (j) DBU, pyridine, 98%; (k) NaOH, EtOH, 90%; (l) DHP, TsOH, 75%; (m) *m*CPBA, $CHCl_3$, 85%; (n) (i) $(PhSe)_2$, $NaBH_4$; (ii) H_2O_2, 63%; (o) Ac_2O, pyridine, 97%; (p) TsOH, AcOH, MeOH, 59%; (q) OsO_4, pyridine, 87%; (r) 2,2-DMP, TsOH, DMF, 84%, (s) $SOCl_2$, pyridine, 58%; (t) TsOH, $CHCl_3$, CH_3OH, H_2O.

Scheme 3. First racemic synthesis of lycoricidine.

The first enantioselective synthesis of (+)-lycoricidine was performed by Paulsen and Stubbe.[31] Enantioselectivity was achieved by utilising a chiral pool starting material, namely a derivative of glucose, aldehyde **50**, Scheme 4. This aldehyde was transformed into nitroolefin **51** and reacted with the lithium derivative of piperonylic ester **52** to produce advanced intermediate **53**. Upon acidic deprotection it was cyclized to cyclohexane **54**, whereupon reduction and recyclization led to 7-deoxypancratistatin (**11**).

This natural product was submitted to selective reprotection followed by elimination to produce lycoricidine (**10**).



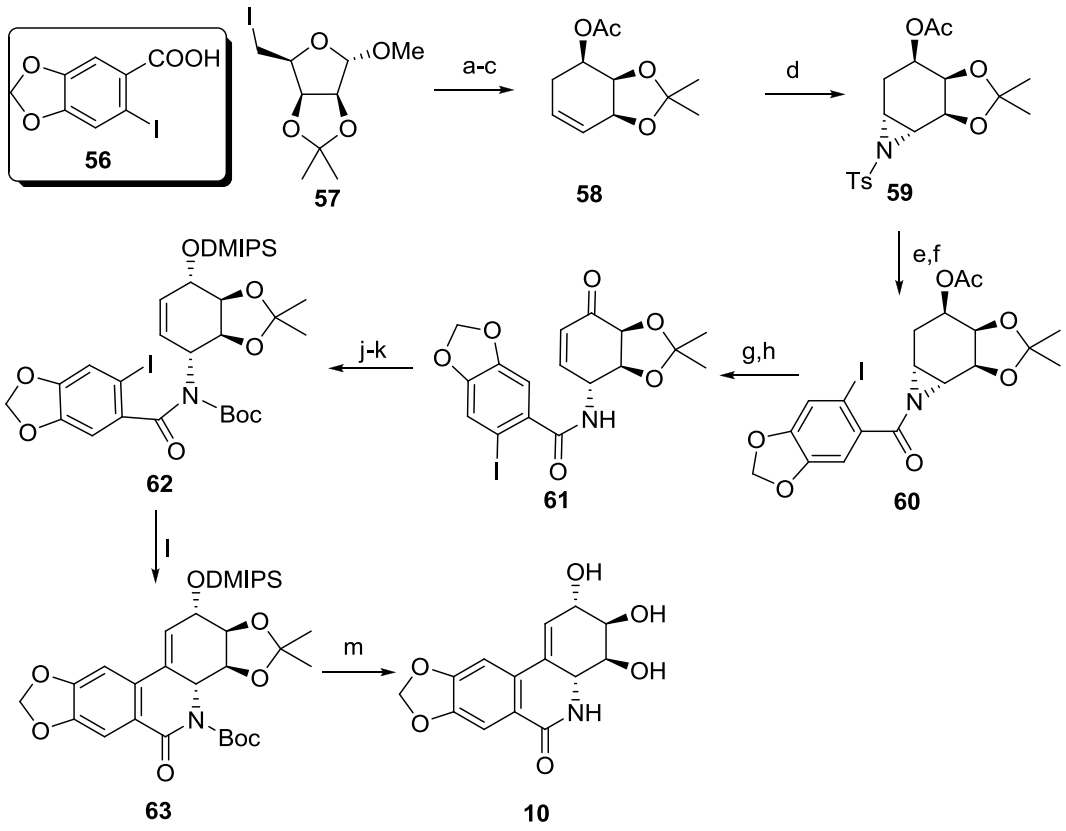
Reaction conditions: (a) (i) CH_3NO_2, NaOH; (ii) Ac_2O, TsOH, 75%; (b) K_2CO_3, benzene, 71%; (c) **52**, *n*BuLi, THF ; (d) (i) AcOH, H_2O; (ii) $NaHCO_3$, MeOH, 34%; (e) H_2, Pd/C, MeOH, 77%; (f) K_2CO_3, MeOH, 72%; (g) BzCl, pyridine, DMAP; (h) $SOCl_2$, pyridine; 70% for 2 steps; (i) NH_3, MeOH.

Scheme 4. First enantioselective synthesis of lycoricidine.

Other syntheses of lycoricidine were reported by Chida in 1991,[32, 33] Hudlický in 1992,[34, 35] by Martin in 1993,[36] by Keck in 1996,[27, 37] and Yan in 2002.[38]

The latest synthesis of lycoricidine was performed by Yadav in 2009.[39]In this chiral pool approach D-(+)-mannose was transformed to ω-iodo glycoside **57** in five steps, as shown in Scheme 5. Iodoether **57** was opened with allyl bromide in presence of zinc and the product of this transformation was cyclized with Grubbs 1st generation catalyst to furnish the cyclohexenol acetate **59**. This product was subjected to two different sets of

aziridination conditions in order to produce the key aziridine **59**. Coupling of 6-iodopiperonylic acid **56** with compound **59** led to *N*-acylated aziridine **60**. Deprotection and oxidation was followed by silica gel-catalyzed rearrangement of the aziridine moiety to allyl amide product **61**. The tertiary amide **62** was subjected to an intramolecular Heck cyclization. This cyclization has been featured in many approaches of lycoricidine, namely those by Ogawa,[32] Hudlický,[34] Martin,[36] and Weinreb.[40] Isocarbostyril **63** was deprotected with formic acid to provide lycoricidine (**10**).

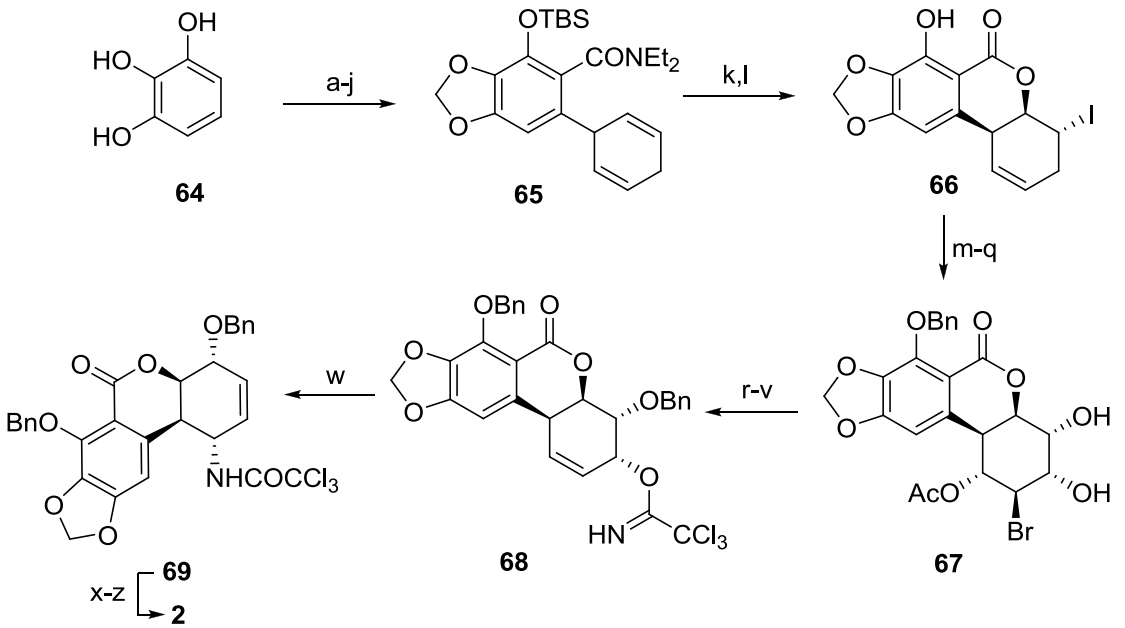


Reaction conditions: (a) allyl bromide, Zn, THF, H_2O, 85%; (b) Grubbs I, CH_2Cl_2, 84%; (c) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 92%; (d) PhINTs, $Cu(acac)_2$, MeCN, 52%; (e) Na/naphthalenide, DME, 67%; (f) **56**, EDCI, CH_2Cl_2, Et_3N, 85%; (g) K_2CO_3, MeOH, 92%; (h) (i) DMP,CH_2Cl_2; (ii) silica gel, 82%; (j) (i) $CeCl_3$, $NaBH_4$, MeOH; (ii) imidazole, DMIPSCl,CH_2Cl_2 90% *dr* 60:40; (k) Boc_2O, DMAP, Et_3N, MeCN, 95%; (l) $Pd(OAc)_2$, Tl(OAc), dppe, anisole, 35%; (m) HCOOH, THF, H_2O, 95%.

Scheme 5. Yadav`s synthesis of lycoricidine.

2.1.2.3. Pancratistatin total syntheses.

Pancratistatin (**2**) has attracted much more attention from the synthetic community than other members of the Amaryllidaceae family. The first total synthesis of **2** was reported by Danishefsky and Lee[41] in 1989 only five years after its isolation. The synthesis began with pyrogallol **64** as the precursor for the A-ring of **2**, Scheme 6. Further functionalization provided amide **65** with all carbons required for the skeleton of pancratistatin. Iodolactonization of cyclohexene ring was performed followed by key installation of the nitrogen atom *via* Overmann rearrangement of compound **68**, lactone **69** was produced. This compound was submitted to dihydroxylation, followed by rearrangement of lactone to amide, and finally, upon the removal of protecting groups, pancratistatin (**2**) was attained.

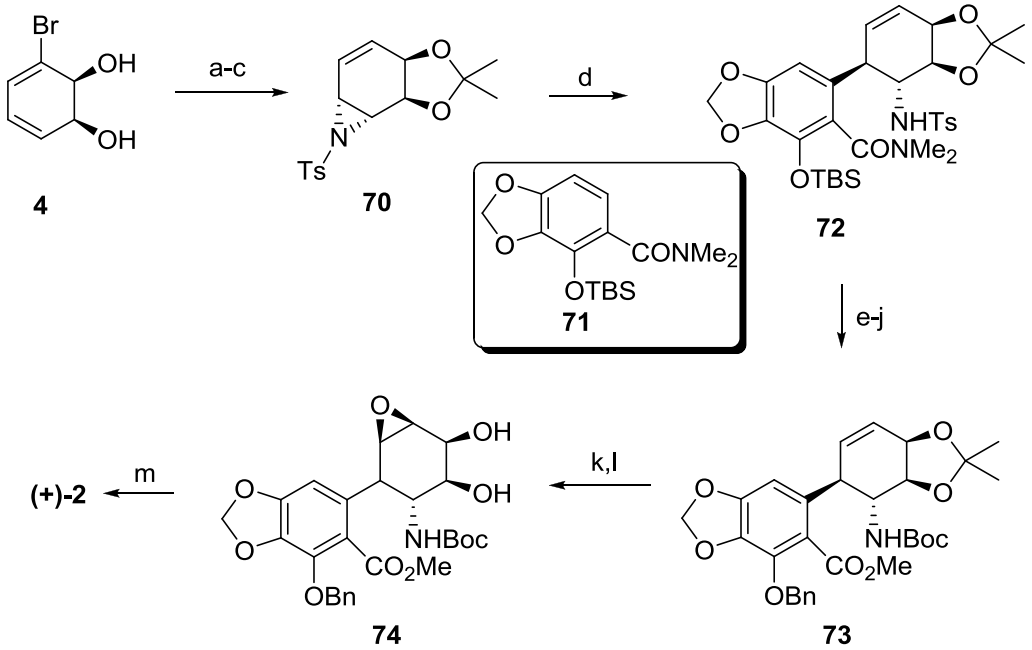


Reaction conditions: (a) $HC(OEt)_3$, amberlyst-15, benzene, 86%; (b) (i) NaH, Et_2NCOCl, THF (ii) MeOH, TsOH, 86%; (c) K_2CO_3, CH_2Br_2, CuO, DMF, 70%; (d) *s*BuLi, TMEDA, THF, 58% (e) TBSCl, imidazole, CH_2Cl_2, 86%; (f) *s*BuLi, TMEDA, THF, 70%; (g) allylmagnesium bromide, Et_2O, 92%; (h) (i) MsCl, Et_3N, CH_2Cl_2; (ii) DBU, 54%; (j) (i)1-(benzenesulfonyl)-2-nitroethene, $CHCl_3$, 96%; (ii) *n*Bu_3SnH, AIBN, PhMe, 72%; (k) TBAF,THF, 79%; (l) (i) (*n*$Bu_3Sn)_2O$, PhMe, (ii) I_2, THF, 67%; (m) BnBr, Ag_2O, DMF, 85%; (n) OsO_4, NMO, CH_2Cl_2 THF, H_2O, 90%; (o) DBU, benzene, 88%; (p) 2-acetoxyisobutyrylbromide, MeCN, 88%; (q) OsO_4, NMO, CH_2Cl_2, THF, H_2O, 88%; (r) *n*Bu_2SnO, PMBBr, PhMe, *n*Bu_4NI, 84% (s) BnBr Ag_2O, DMF, 95%; (t) DDQ, CH_2Cl_2, H_2O, 75%; (u) Zn, AcOH, H_2O, CH_2Cl_2, 81%; (v) NaH CCl_3CN, THF, 74%; (w) 100°C, hich-vac, 56%; (x) OsO_4, NMO, THF, H_2O, 75%; (y) (i) K_2CO_3 MeOH,CH_2Cl_2; (ii) amberlyst-15; (iii) DCC, 82%; (z) H_2, $Pd(OH)_2$, EtOAc, 90%.

Scheme 6. Danishefsky`s synthesis of pancratistatin.

The first enantioselective synthesis was reported by Hudlický[42, 43] in 1995. Chirality in this synthesis was provided by the microbial metabolite *cis*-diol **4**, Scheme 7. It was transformed into vinyl aziridine **70**, which was subjected to nucleophilic opening with the cuprate derived from *ortho*-lithiation of amide **71**. This sequence provided compound **72**, containing all carbons of (**2**). Cyclization of **72** upon detosylation should have provided short access to the phenanthridone skeleton of the final target, but because of atropoisomerism the cyclization of amide did not take place and no desired phenanthridone was obtained. Therefore the expected short access to the major skeleton

was somewhat hampered by lengthy functionalization steps in order to provide the desired phenanthridone. The endgame was achieved by heating epoxide **74** in water, providing pancratistatin in 13 steps.



Reaction conditions: (a) 2,2- DMP, TsOH, acetone; (b) PhI=NTs. $Cu(acac)_2$, MeCN, 27% for 2 steps; (c) $n$$Bu_3SnH$, AIBN, THF, reflux. 78%; (d) (i) **71**, sBuLi, TMEDA, THF; (ii) CuCN, (iii) **70**, BF_3*Et_2O, 49%; (e) (i) sBuLi, THF; (ii) $(Boc)_2O$, 68%; (f) Na/anthracene, DME, 62%; (g) SMEAH, morpholine, THF, 72%; (h) BnBr, K_2CO_3, DMF, 83%; (j) (i) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, tBuOH, H_2O; (ii) CH_2N_2, 98%; (k) HOAc, THF, H_2O, 73%; (l) tBuOOH, $VO(acac)_2$, benzene, 53%; (m) $PhCO_2Na$, H_2O, reflux, 51%.

Scheme 7. Hudlický`s enantioselective synthesis of pancratistatin.

This report was closely followed by that of Trost who reported another synthesis of (**2)** in the same year.[44] The next formal synthesis was performed by Haseltine in 1997,[45]and Magnus and Sebhat completed their total synthesis in 1998.[46, 47] Rigby utilized his previously used narciclasine strategy for the synthesis of pancratistatin in 2000.[24] A relay synthesis of pancratistatin from narciclasine was published in 2001 by Pettit[48] and will be discussed in detail in section 2.1.3.4. This approach was followed by Kim`s synthesis in

2002.[49, 50] In 2006 Li[51] completed one of the shortest synthesis of pancratistatin. All of these early syntheses of (**2**) up to 2008 have been covered in an excellent review[13] by Kornienko and Manpadi along with the estimation of economic viability of each route.

In 2009 Madsen applied a chiral pool strategy for the synthesis of pancratistatin (**2**).[52] The chief strategy rests on the reaction of methyl ω-iodoglycoside **82** with allyl bromide **78** in the presence of zinc, followed by ring-closing metathesis,

Figure **9**. Allyl bromide **78** was produced from piperonal **75** in 10 steps. Lactone **80** was obtained from **79** by ring-closing metathesis. At this point, a formal total synthesis of pancratistatin was achieved by intercepting the intermediate reported by Danishefsky.[41] Nevertheless, the endgame of the synthesis was improved in comparison with the original sequence by utilisation of milder conditions.

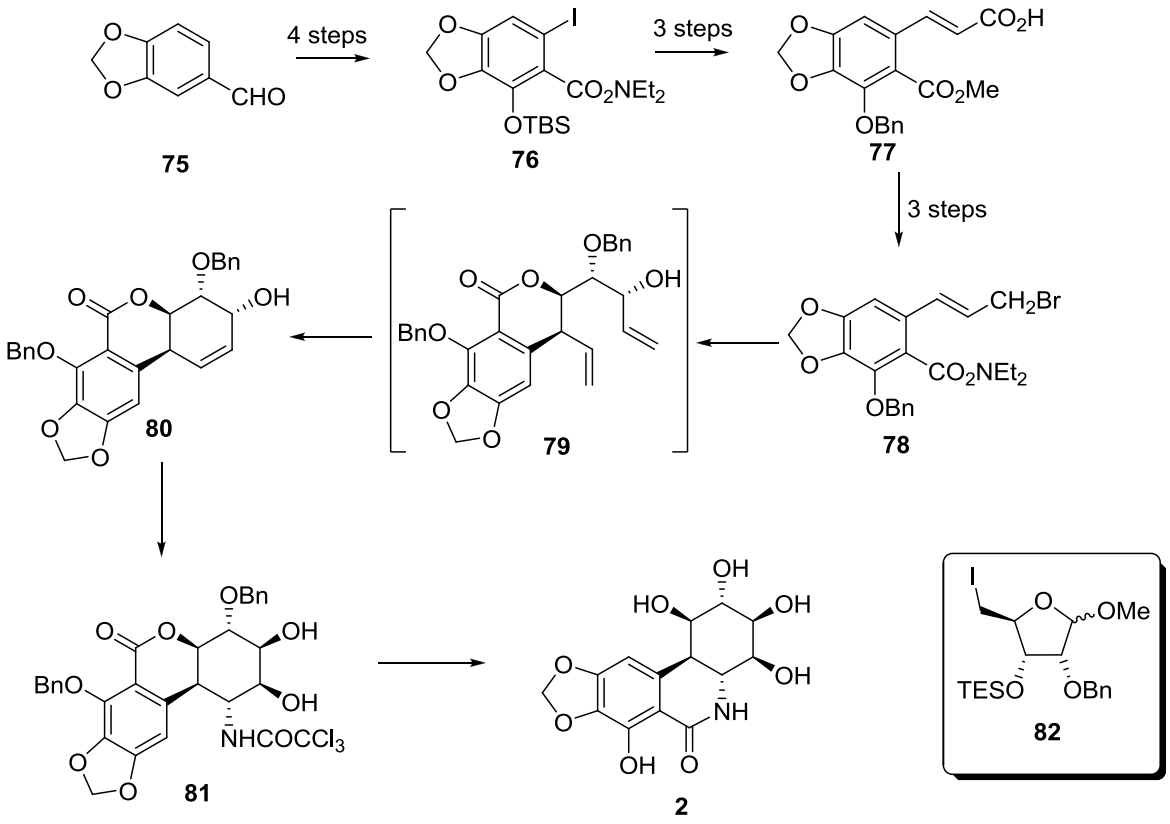


Figure 9. Madsen`s synthesis of pancratistatin.

The most recent enantioselective synthesis of pancratistatin was published by Alonso.[53] The main strategy of Alonso`s approach lies in the organocatalytic condensation of β-aryl-α-nitro-α,β-enals such as **85** with protected dihydroxyacetone **83** in the presence of pyrrolidine catalysts, Figure 10. Enal **85** was obtained from 5-methoxypiperonal **84** in two steps. Formal [3+3] annulation reaction of **85** with protected dihydroxyketone **83** provided the key intermediate **86** with five contiguous chiral centres identical to those of final target. The optical purity of **86** was improved up to 99:1 *er* by a single recrystallization and **86** was then subjected to subsequent reduction, reprotection and Bischler-Napieralski cyclization to provide phenanthridone **89**. Deprotection of **89** furnished (**2**), accessed in nine one-pot operations. This strategy was proven to be

versatile and was applied to the synthesis of 7-deoxypancratistatin and a few analogues that will be discussed in the Section 2.1.3.3.

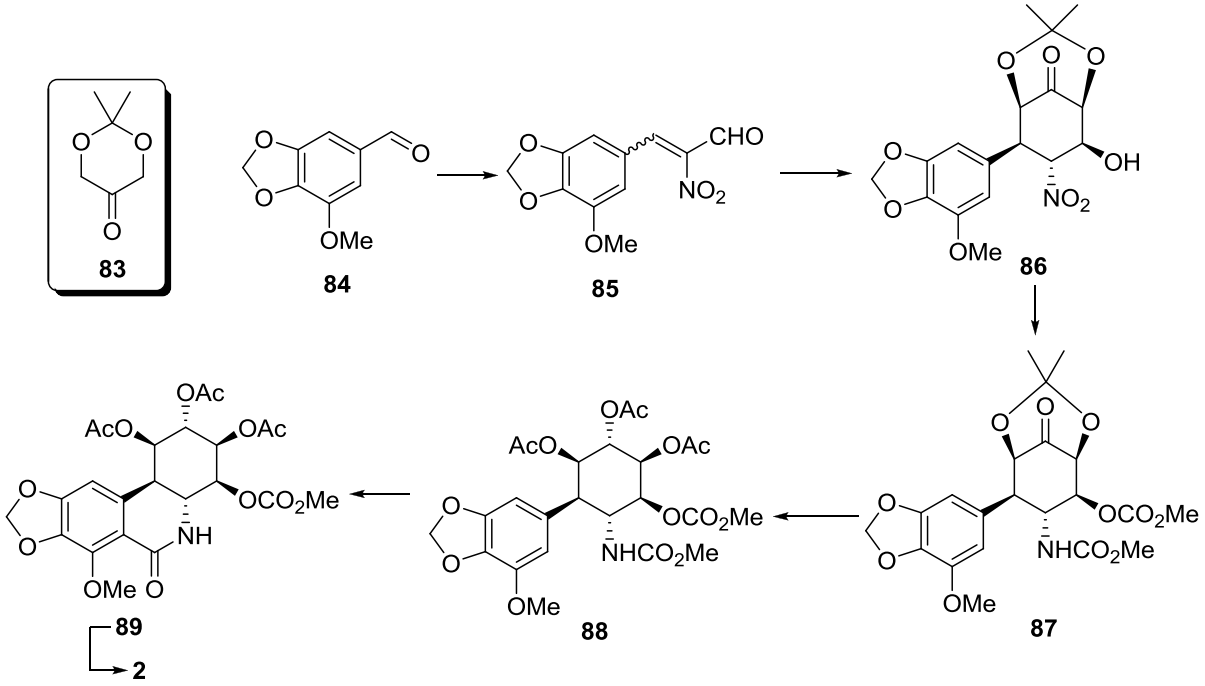
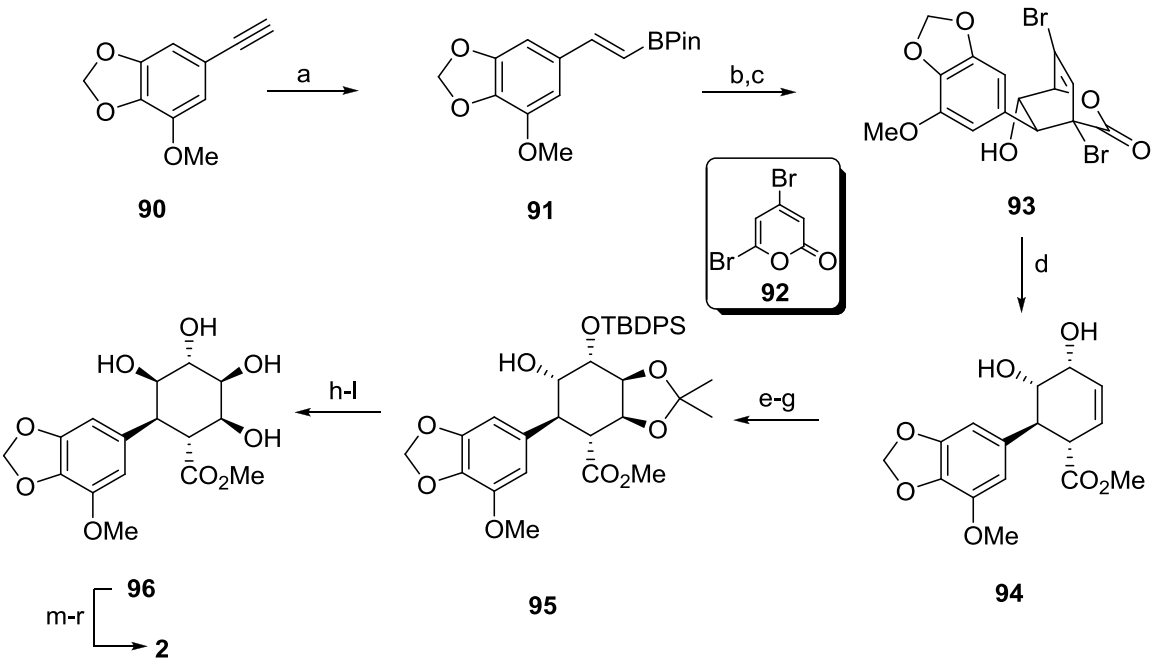


Figure 10. Alonso`s synthesis of pancratistatin.

The most recent racemic synthesis of pancratistatin has been performed by Cho *et al.*[54] This synthesis followed they previously described strategy, which was already utilized once for the synthesis of pancratistatin (**2**) in 2011.[55] It employed a Diels-Alder reaction between 3,5-dibromo-2-pyrone (**92**) and styrene **91**, Scheme 8. Since those two syntheses are strategically similar, only the latest one will be discussed. The synthesis began with *trans*-borylation of alkyne **90**, thus affording alkene **91**, which underwent the required [4+2] cycloaddition with **92**. Oxidation of boron yielded bicyclic product **93** which was reduced and ring-opened to provide diol **94**, selective protection and dihydroxylation of which provided the skeleton of 1-*epi*-pancratistatin **95**. Inversion of the C-1 center provided tetraol **96**, which was submitted to a sequence previously developed by this

group, that included Curtius rearrangement and Bischler-Napieralski cyclization to yield pancratistatin (**2**), Scheme 8.



Reaction and conditions: (a) $B_2(Pin)_2$, Cp_2ZrHCl, CH_2Cl_2, 82%; (b) **92**, PhMe, 86%; (c) $NaBO_3$, THF, H_2O, 81%; (d) (i) Zn, NH_4Cl, H_2O; (ii) TsOH, MeOH, 52%; (e) TBDPSCl, imidazole, CH_2Cl_2, 88%;(f) OsO_4, NMO, THF, H_2O, 100%; (g) 2,2-DMP, CSA, 93%; (h) DMP, CH_2Cl_2, 95%; (j) $NaBH_4$, MeOH, 100%; (k) AcOH, H_2O, 100%; (l) TBAF, THF, 100%; (m) LiOH, THF, 100%; (n) (i) DPPA, Et_3N, PhMe; (ii)NaOMe, MeOH, 86%; (o) Ac_2O, pyridine; (p) Tf_2O, CH_2Cl_2, DMAP, 60 % for 2 steps; (q) BBr_3,CH_2Cl_2, 70%; (r) NaOMe,THF, 90%.

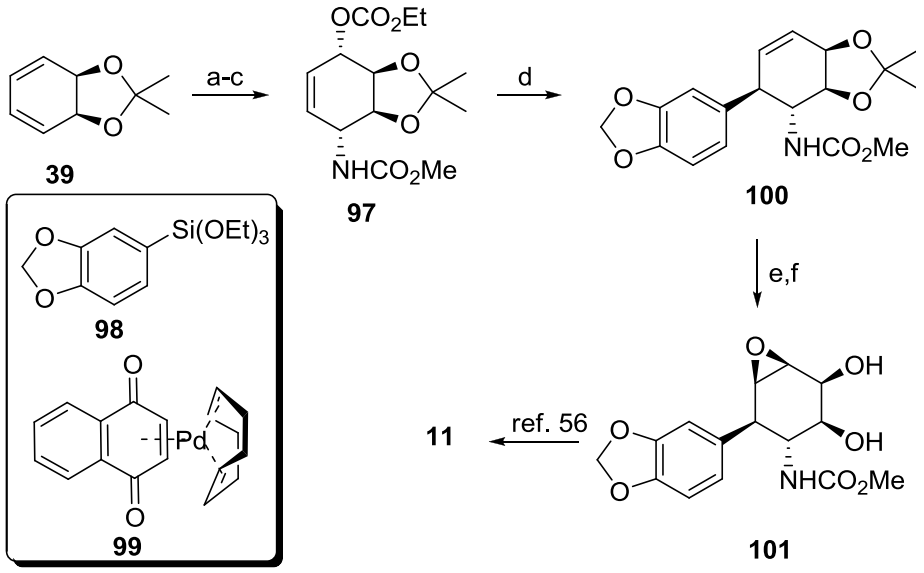
Scheme 8. Cho`s synthesis of pancratistatin.

2.1.2.4. Total syntheses of 7-deoxypancratistatin.

Since the first enantioselective total synthesis of **11** was performed even before its isolation from a natural source in 1989, as part of the synthesis of lycoricidine, Scheme 4, it will not be reproduced in this section. A list of syntheses performed after its isolation consists of enantioselective synthesis by Hudlický in 1995,[56] which was realised through a strategy similar to that for pancratistatin, described by this group previously.[42] This synthesis was closely followed by a radical approach by Keck in 1995.[57] One year later

Chida[58] utilised a chiral pool approach, followed by Plumet in 2000,[59] who made use of the nucleophilic opening of vinylsulfones. A combination of ring closing metathesis and allyl coupling was utilised by Madsen in 2006,[60] and Padwa reported a racemic synthesis in 2007[61] making use of an intramolecular Diels-Alder reaction.

The latest formal total synthesis of racemic 7-deoxy pancratistatin (**11**) was performed by DeShong and Shukla[62] in 2012. Crucial connectivity in **100** was established by coupling of a π-allyl complex formed from protected conduramine **97** with siloxane **98**, Scheme 9. Preparation of the C-ring fragment ensued from the protected diol **39**, previously used in the synthesis of narciclasine (**1**) by Yan.[28] A hetero-Diels-Alder reaction was performed on **39**, followed by reduction and protection to provide the key intermediate **97** which was then submitted to Hiyama coupling with silane **98** in the presence of palladium catalyst **99**, which yielded product **100** with moderate yield. Further cylization and oxidation, described previously by Hudlický,[56] led to epoxide **101,** an intermediate in the aforementioned synthesis.



Reaction and conditions: (a) $NH(OH)CO_2Me$, nBu_4IO_4, $CHCl_3$/DMF, 31%; (b) $Mo(CO)_6$, MeCN, H_2O, 79%; (c) $ClCO_2Et$, pyridine, CH_2Cl_2, 86%; (d) **98**, **99**, TBAF, THF, 35%; (e) AcOH, THF, H_2O, 94%; (f) *t*BuOOH, $VO(acac)_2$, MeCN, 85%.

Scheme 9. DeShong`s formal synthesis of 7-deoxypancratistatin.

2.1.3. Synthesis of analogues of Amaryllidaceae alkaloids

Promising anticancer activity and the as-yet unresolved mechanism of the biological action of isocarbostyril congeners of Amaryllidaceae family has attracted the interest of the synthetic organic and biological community alike. One of the major obstacles on the way to a marketable drug is the poor aqueous solubility of isocarbostyril compounds. This problem can be somewhat compensated for by the preparation of water–soluble prodrugs such as phosphates, however, the relatively low availability of Amaryllidaceae congeners from natural sources has ensured that significant efforts have been directed towards total synthesis. Unfortunately, because of the complexity of targets, existing approaches are somewhat lengthy (12-18 steps) and suffer from impracticality for large scale preparation, given the overall yields of 2-7%. The few syntheses with the higher yields do not provide the necessary flexibility to perform variation in different parts of

the target compounds in order to generate libraries of analogues. Therefore there are three goals that the syntheses of analogues of the aforementioned compounds need to target: (i) to establish the minimal pharmacophore required for anticancer activity and elucidate the mode of action, (ii) to produce water-soluble version of these compounds, and (iii) to devise short and practical routes to biologically active compounds.

There are currently two general approaches to the analogues: the first one is the modification of the most abundant natural congener of this family – narciclasine (**1**). This approach was extensively developed by Pettit, the discoverer of pancratistatin. The attractiveness of such a route to study the pharmacophore lies in the relatively fast access to the desired compounds. This approach will be discussed in a Section 2.1.3.4.

The second approach, which is somewhat related to total synthesis effort, is to produce fully synthetic analogues of Amaryllidaceae congeners from commercially available starting materials. This route allows an approach to analogues that cannot be produced by modification of natural congeners and does not rely on limited natural sources. The analogues can be divided into three main groups depending on where the major structural differences to the natural products are located. In the Section 2.1.3.1, synthetic analogues with modification in C-ring will be discussed, followed by synthetic analogues of the B-ring, Section 2.1.3.2, and A-rings analogues, Section 2.1.3.3.

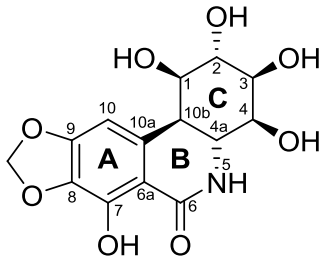


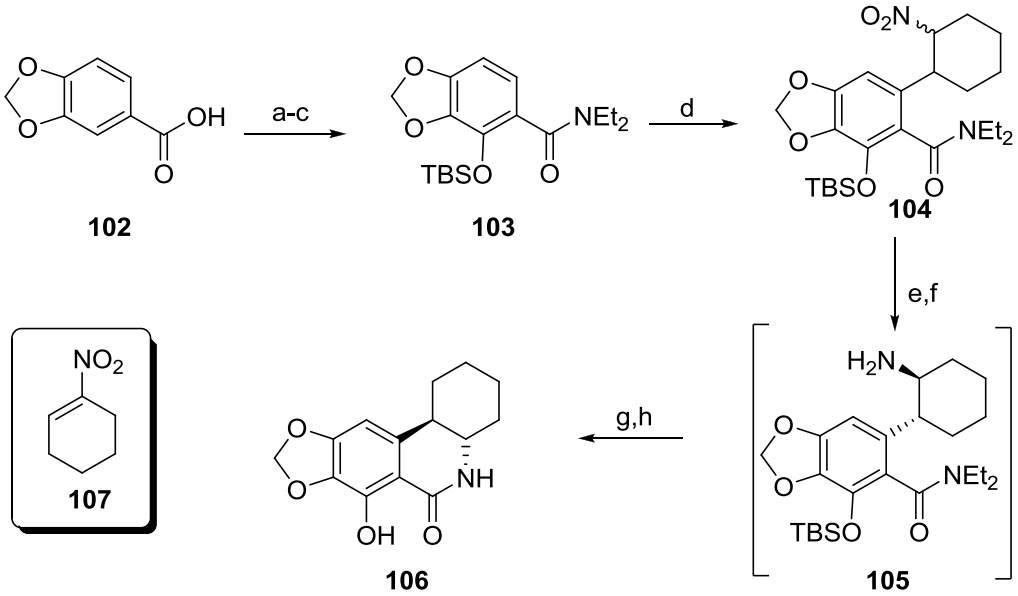
Figure 11. Narciclasine/Pancratistatin nomenclature and numbering.

2.1.3.1. C-ring analogues

The most explored class of analogues possess variations in the ring C. Because of the inherent complexity, namely four contiguous hydroxylated stereogenic centers, it only seems logical to explore deoxy analogues as well as compounds with different relative stereochemistry of the hydroxyl groups in order to establish a minimum pharmacophore.

One of the first synthetic analogues of **2** with a completely deoxygenated C-ring was synthesized by Heathcock.[63] The main strategy involved the Michael-type 1,4-addition of ortho-metalated amide **103** to the 1-nitrocyclohexene **107** to produce aryl nitrocyclohexane **104**, Scheme 10. Piperonilic acid **102** was converted into its diethylamide, selectively *ortho*-lithiated, borylated, oxidised, and protected to produce amide **103**. The latter was in turn *ortho*-metalated and the resulting aryl lithium was added to **107** to produce the mixture of *cis*- and *trans*- stereoisomers **104** which was epimerised to the *trans*-isomer and reduced to the corresponding amine **105**. It was cyclized by treatment with *s*-BuLi and deprotected to produce **106** in seven steps. Unfortunately, this model compound was not subjected to any biological studies. This approach was supposed to be a model for the actual synthesis of **2**, but it is worth to note

that such strategy failed to produce the desirable outcome, transamidation of arylamide with the amino group, when it was later applied to the synthesis of pancratistatin.[42]

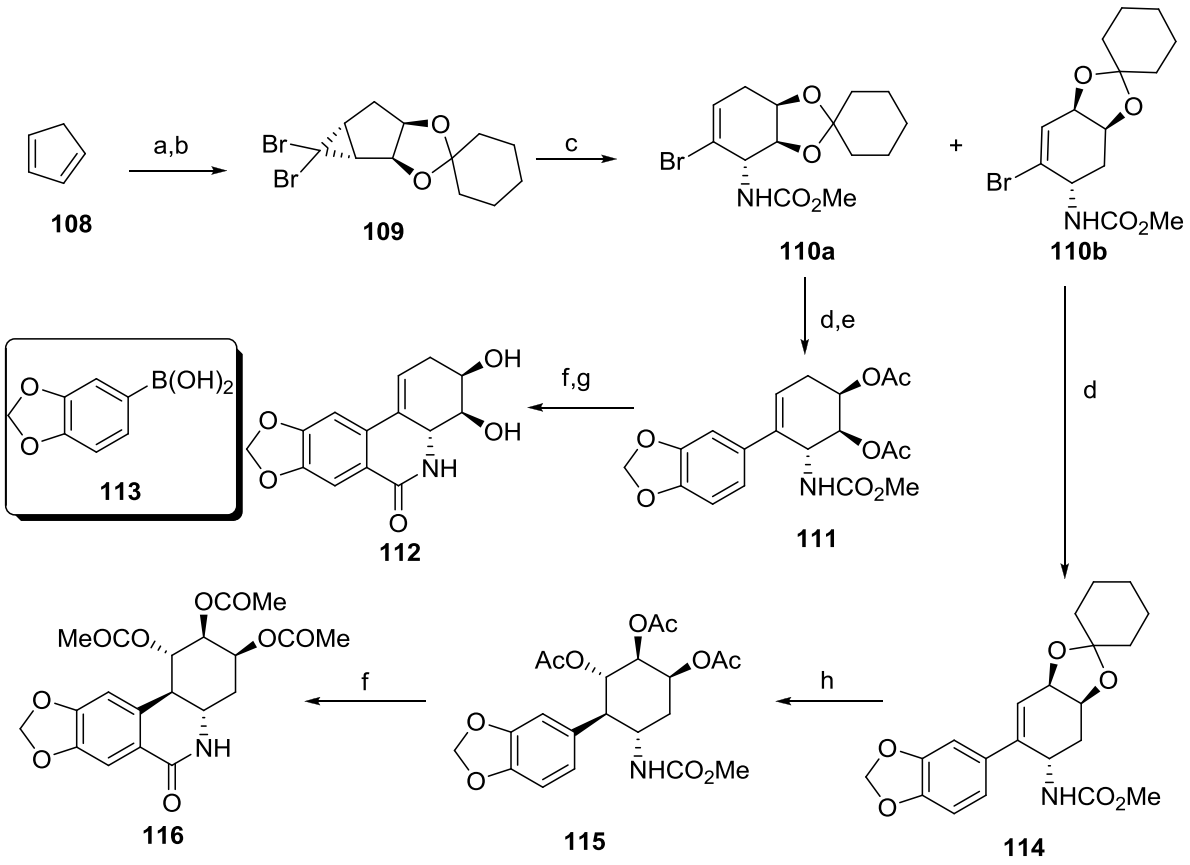


Reaction conditions: (a) (i) $SOCl_2$; (ii) Et_2NH, Et_2O, 71%; (b) (i) *s*-BuLi, TMEDA, THF; (ii) $B(OMe)_3$; (iii) H_2O_2, AcOH; (c) TBSCl, imidazole,CH_2Cl_2 80 % for 2 steps; (d) (i) *s*-BuLi, TMEDA, THF; (ii) **107**; (iii) AcOH 70%; (e) Et_3N, EtOH, 83% (f) $NaBH_4$, $NiCl_2$*$6H_2O$, MeOH,))); (g) *s*-BuLi, THF; (h) HCl 47% for 3 steps.

Scheme 10. Heathcock`s synthesis of a pancrastistatin model.

Banwell[64] developed a new way to analogues of narciclasine and pancratistatin while exploring approaches to the synthesis of the skeleton of narciclasine itself. The key strategy in his synthesis lies in a Suzuki coupling between boronic acid **113** and functionalised 1-bromocyclohexenes **110a** and **110b**, Scheme 11. The synthetic sequence started with dihydroxylation of cyclopentadiene **108** with $Pb(OAc)_4$ followed by protection of the diol, and addition of dibromocarbene led to the formation of **109**. This dibromocyclopropane was rearranged upon reaction with silver isocyanate and trapped with methanol to produce a mixture of isomeric bromocyclohexenes **110a** and **110b** (in 28% and 40% yield respectively), both of which could be elaborated to natural product

analogues. These compounds were separated and subjected to Suzuki coupling with boronic acid **113**. Carbamate **111** was obtained by acetate reprotection after Suzuki coupling of **110a** and was subjected to Bischler-Napieralski cyclization with triflic anhydride and further deprotected by sodium methoxide to produce 2,7-dideoxynarcilasine (**112**) in six steps. Vinylbromide **110b** was subjected to the same sequence to produce alkene **114**. This isomer was stereoselectively borylated (as a result of steric hindrance of ketal group), oxidised, and reprotected as a triacetate to produce carbamate **115**. This compound was submitted to the Bischler-Napieralski reaction to furnish triacetate of 1,2-*epi*-4,7 dideoxypancratistatin (**116**). Banwell`s strategy thus allowed for the synthesis of analogues of narciclasine and pancratistatin utilizing the same sequence and starting materials.

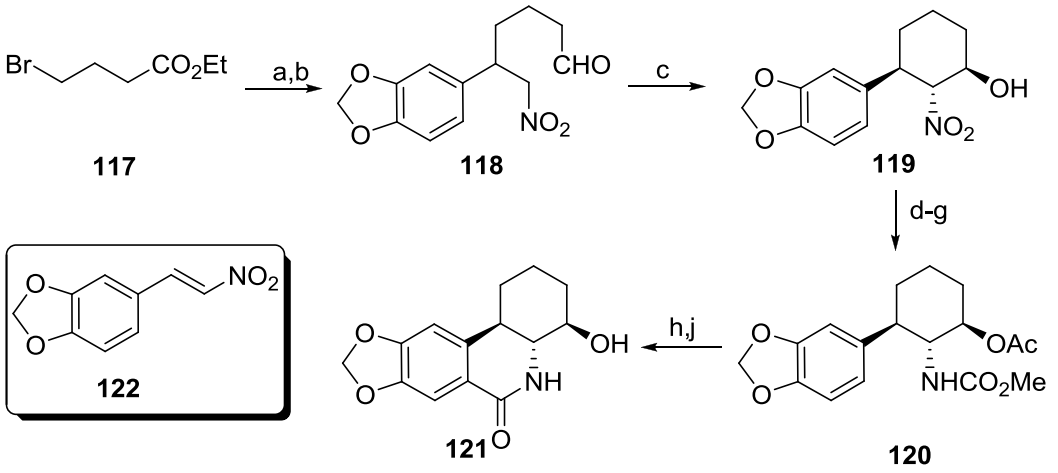


Reaction conditions: (a) (i) $Pb(OAc)_4$, AcOH; (ii) K_2CO_3, MeOH; (iii) cyclohexanone, TsOH, 70%; (b) $CHBr_3$, NaOH, water/benzene, 84%; (c) (i) AgOCN,, dioxane; (ii) NaOMe, MeOH, 28% **110a** 40% **110b**; (d) $Pd(PPh_3)_4$, benzene, EtOH, 2M Na_2CO_3, 82% **111**, 87% for **111**; (e) (i) HCl, THF; (ii) Ac_2O, DMAP, pyridine, CH_2Cl_2, 89%; (f) Tf_2O, DMAP, CH_2Cl_2, 85% for **112**, 59% for **110**; (g) NaOMe, MeOH-THF, 96%; (h) (i) BH_3*THF; (ii) NaOH, H_2O_2; (iii) HCl, THF; (iv) Ac_2O, DMAP, pyridine, CH_2Cl_2, 42% .

Scheme 11. Banwell`s synthesis of 2-deoxynarciclasine and 1-*epi*-4-deoxypancratistatin.

Significant efforts toward the refinement of the C-ring pharmacophore have been reported by McNulty,[65-67] whose systematic study led to the recognition of nitrostyrene **122** as a common intermediate for the synthesis of several different racemic deoxy analogues. The first approach was based on a stereoselective intramolecular aldol condensation of nitroaldehyde **118**,[67] Scheme 12. Addition of an organometallic reagent generated from ethyl 4-bromobutyrate **117** to nitrostyrene **122** which upon reduction led

to aldehyde **118.** Submission of this intermediate to solid-phase condensation in the presence of neutral alumina yielded nitrocyclohexanol **119** with good stereoselectivity (95:5). A series of protection and reduction transformations afforded carbamate **120** which, after Bischler-Napieralski cyclization and deprotection, afforded 2,3-dideoxy *trans*-dihydrolycoricidine (**121**) in 8 steps.

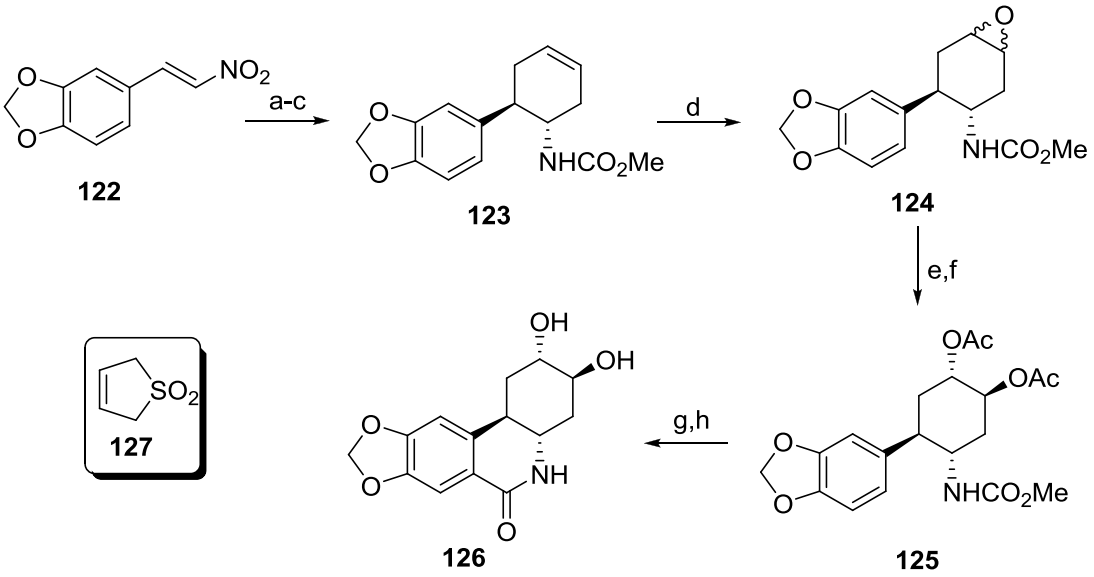


Reactions conditions: (a) (i) Zn, LiI, DMF; (ii) CuCN, LiCl, THF; (iii) **122**, 81%; (b) DIBAL, CH_2Cl_2; (c) Al_2O_3, 48h, 71% for 2 steps; (d) TBSCl, imidazole, DMF, 96%; (e) H_2, 43 atm, Raney Ni, MeOH, 100%; (f) $ClCO_2Me$, Et_3N, CH_2Cl_2, 96%; (g) Ac_2O, $FeCl_3$, 85%; (h) (i) Tf_2O, DMAP, CH_2Cl_2; (ii) HCl, THF, (iii), Ac_2O, DMAP, Et_3N, 85%; (j) NaOMe, THF, MeOH, 96%.

Scheme 12. McNulty`s synthesis of 2,3-dideoxy *trans*-dihydrolycoricidine.

Latter approaches by McNulty towards the construction of deoxy analogues were based on a Diels-Alder reaction between the aforementioned nitrostyrene **122** and different dienes.[66,65] It was shown that this approach can lead to the formation of the essential *trans*-stereochemical relationship between the aryl ring and nitro group. Independently, the same stereoselectivity was reported by Iglesias[68] on the similar systems. Two different dienes were utilised for the synthesis of two analogues of *trans*-dihydrolycoricidine.

For the synthesis of 4-deoxy *trans*-dihydrolycoricidine (**126**), 2,5-dihydrosulfolane (**127**) as a diene precursor was used in a Diels-Alder reaction with nitrostyrene **122** in the presence of Lewis acidic $ZnCl_2$,[66] Scheme 13. The cycloadduct was reduced with aluminum amalgam and transformed to carbamate **123**, which was further epoxidised to a mixture of α- and β-epoxides **124**. Nucleophilic *trans*-diaxial opening of both isomers, followed by acetylation led to the same stereochemical relationship in diacetate **125**. The same result for selective ring opening of similar system was reported three years earlier by Toke.[69] Carbamate **125** was submitted to modified cyclization conditions described by Banwell,[70] and deacetylated to provide 4-deoxy *trans*-dihydrolycoricidine (**126**) in seven steps.

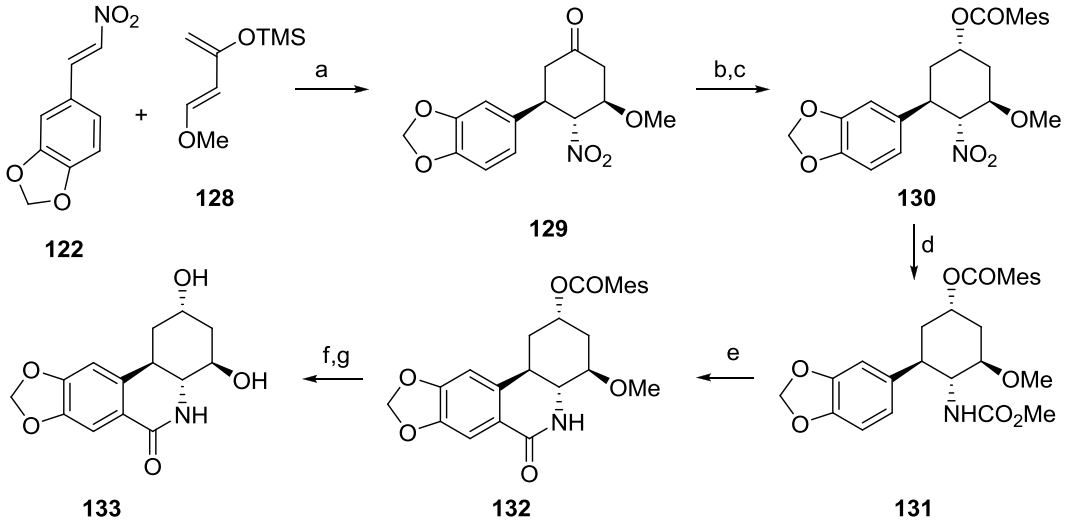


Reaction conditions: (a) **127**, $ZnCl_2$, PhMe, 85%; (b) Al/Hg, THF, H_2O; (c) $ClCO_2Me$, Et_3N, CH_2Cl_2, 96% for 2 steps; (d) *m*CPBA, CH_2Cl_2, 93%; (e) $PhCO_2Na$, H_2O; (f) Ac_2O, pyridine, 62% for 2 steps; (g) (i) Tf_2O, DMAP, CH_2Cl_2; (ii) HCl, dioxane, 65%; (iii) Ac_2O, pyridine, 85%; (h) NaOMe, THF, MeOH, 96%.

Scheme 13. McNulty`s synthesis of 4-deoxy *trans*-dihydrolycoricidine.

In order to make the last analogue **133**, nitrostyrene **122** was subjected to the cycloaddition with Danishefsky`s diene **128** in refluxing toluene to provide

cyclohexanone **129** in good yield, Scheme 14. The ketone was reduced to alcohol with sodium borohydride and converted *via* Mitsunobu reaction to nitrocyclohexane **130**. The nitrogroup was then reduced to carbamate **131**, which was subjected to the Bischler-Napieralski reaction and after demethylation and removal of the ester provided 3- deoxy *trans*-dihydrolycoricidine (**133**).[66]

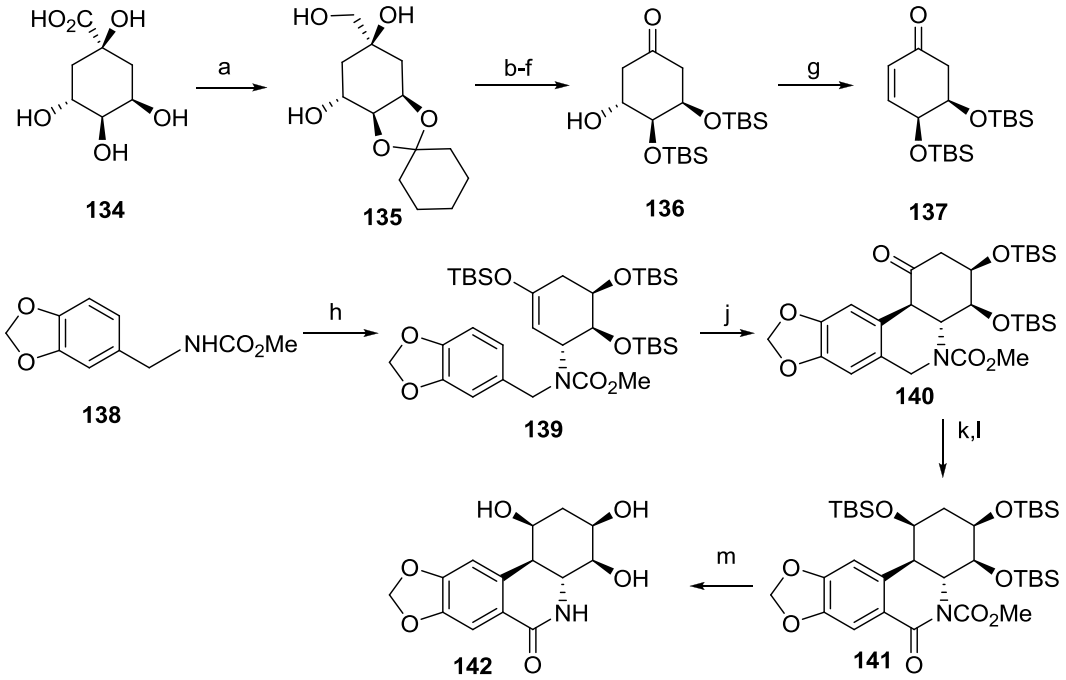


Reaction conditions: (a) PhMe; (b) $NaBH_4$, EtOH; (c) MesCOOH, $Bu_3P{=}C(CO_2Me)_2$, $PhCH_3$, 70% for 3 steps; (d) (i)Al/Hg, EtOH, H_2O; (ii) $ClCO_2Me$, pyridine, CH_2Cl_2,70%; (e)Tf_2O, DMAP, 65%; (f) BI_3, CH_2Cl_2, 15%; (g) $LiAlH_4$, THF.

Scheme 14. McNulty`s approach to 3-deoxy analogues of *trans*-dihydrolycoricidine.

Pandey[71] reported an enantioselective photochemical approach towards pancratistatin analogues. His synthesis started from D-quinic acid **134**, which was transformed into ketal **135** and subjected to several protecting group manipulations, followed by sodium periodate cleavage of unprotected diol moiety to produce hydroxy ketone **136**, Scheme 15. A mesylation-elimination sequence of the free hydroxyl produced enone **137**, which served as the C-ring component in this synthesis. Michael addition of carbamate **138** to this enone followed by silyl protection of the enol led to key intermediate **139**, which in

turn was submitted to photocyclization in the presence of 1,4-dicyanonaphthalene (DCN). This transformation established the necessary *trans* ring juncture in tricycle **140**. Steric hindrance of the β-face resulted in selective reduction of the carbonyl to establish the correct stereochemistry in the C-ring of the product. Further protection and ruthenium-catalyzed oxidation of the benzylic position afforded amide **141**, which after protection yielded the final product 2,7-dideoxypancratistatin (**142**).

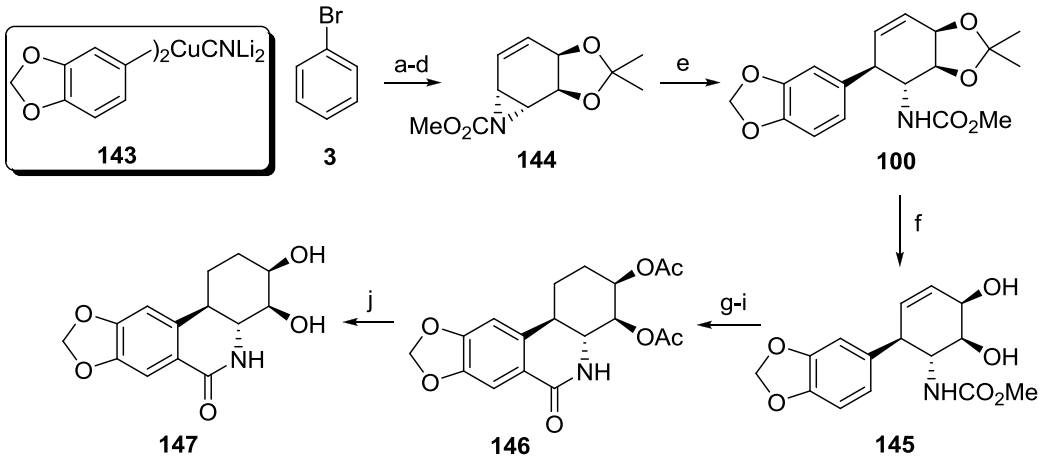


Reaction conditions: (a) (i) cyclohexanone, Amberlyst-120; (ii) $NaBH_4$, EtOH 60% for 2 steps; (b) NaH, BnCl, DMF, 85%; (c) AcOH-H_2O, 95%; (d) TBSCl, imidazole, DMF, 80%; (e) H_2, Pd/C, EtOH, quant.; (f) $NaIO_4$, EtOH, 95%; (g) MsCl, Et_3N, CH_2Cl_2, 95%; (h) (i) **138**, *n*BuLi, HMPA, THF; (ii) TBSCl, 95%; (j) hν, DCN, CH_3CN, H_2O, 68%; (k) (i) $NaBH_4$, *i*PrOH; (ii) TBSCl, imidazole, DMF, 85%; (l) RuO_2, $NaIO_4$, EtOAc, H_2O, 90%; (m) (i) NaOMe, MeOH; (ii) TBAF, THF, 90%.

Scheme 15. Pandey`s synthesis of 2,7-dideoxypancratistatin.

In order to perform the synthesis of a further deoxygenated analogue of 7-deoxypancratistatin,[72] Hudlický utilized the route previously developed in his group towards 7-deoxypancratistatin (**11**),[56] Scheme 16. The synthesis started with whole-cell

oxidation of bromobenzene **3** followed by acetonide protection of the resulting diol, which was converted to aziridine **144** followed by dehalogenation. Nucleophilic opening of this aziridine with cuprate **143** in the presence of a Lewis acid led to amide **100**, which in turn was deprotected to diol **145**. Hydrogenation of alkene moiety with hydrogen on palladium on carbon was followed by Bischler-Napieralski cyclization and base-catalyzed deprotection to provide 1,2,7-trideoxypancratistatin (**147**). Synthesis and biological studies of this compound along with those synthesized by McNulty, (Scheme 13, Scheme 14), completed all possible deoxy models of the C-ring of Amaryllidaceae compounds. Biological results will be discussed in section 2.1.4.

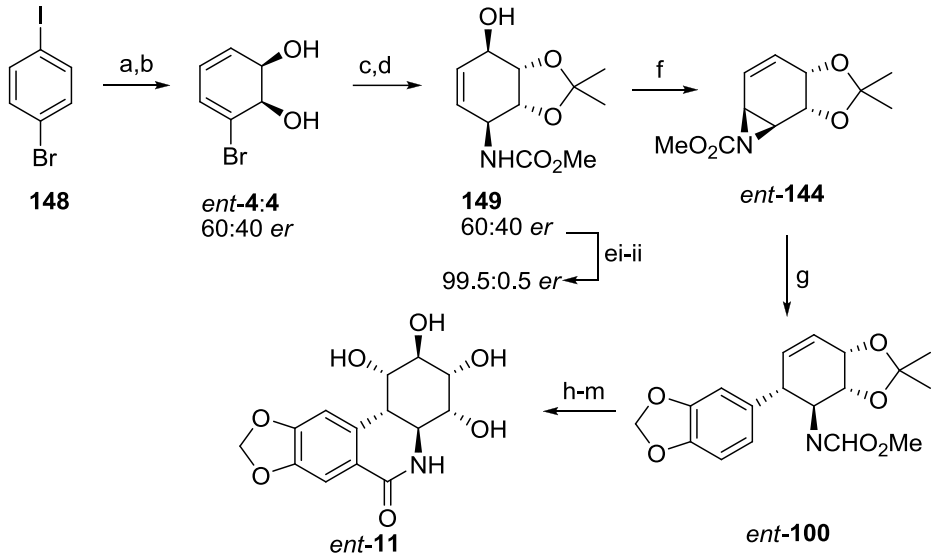


Reaction conditions: (a) *E. Coli* pDTG601; (b) 2,2-DMP, TsOH; (c) $NsONHCO_2Me$, K_2CO_3, CH_2Cl_2, 80%; (d) Bu_3SnH, AIBN, THF, 54%; (e) **143**, BF_3*Et_2O, THF, 34%; (f) AcOH, THF, H_2O, 98%; (g) Ac_2O, pyridine, 98%; (h) H_2 Pd/C, EtOH, 95%; (i) Tf_2O, DMAP, CH_2Cl_2, 50%; (j) NaOMe, MeOH, 70% 56

Scheme 16. Hudlický`s synthesis of 1,2,7-trideoxypanratistatin.

Since lycorine (**8**) showed some activity in anticancer assays and it possesses relative stereochemistry in positions 10b, 1, and 2 (narciclasine numbering) opposite to those of pancratistatin, it seemed reasonable to explore biological activity of the enantiomer of 7-deoxypancratistatin in anticancer assays. In order to synthesize such compounds and

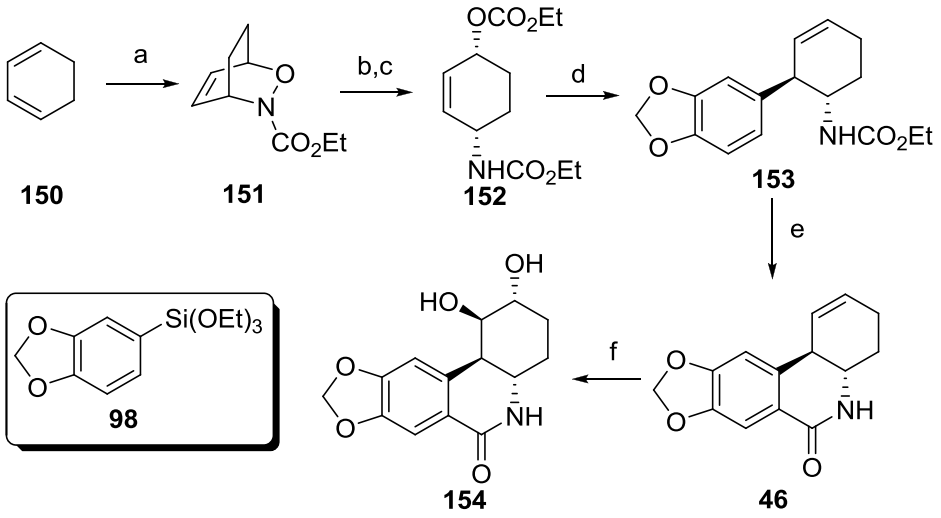
study their activity, Hudlický and Akgun[29] utilised the toluene dioxygenase (TDO) enzyme, which can discriminate between the dihydroxylation of aromatic compounds based on relative size of their substituents. *p*-Bromoidoobenzene **148** was submitted to the whole-cell oxidation and after selective radical reduction provided a mixture of bromocyclohexadienediols **4** and *ent*-**4**, Scheme 17. Submission of this mixture to a nitroso hetero-Diels-Alder reaction and reduction led to a mixture of conduramines **149**, which upon acylation and enzymatic resolution provided enantiomerically pure **149**, Scheme 17. This conduramine was transformed to aziridine *ent*-**144**, the enantiomer of which was successfully utilised before for the synthesis of 7-deoxypancratistatin (**11**).[56] The same conditions were applied in case of this compound to provide *ent*-**11** and enabled the study of its biological activity, which turned out to be one order of magnitude less active that natural congener.



Reaction conditions: (a) *E.Coli* JM109 (pDTG601); (b) nBu$_3$SnH, 55%; (c) (i) 2,2-DMP, TsOH; (ii) HONHCO$_2$Me, NaIO$_4$, MeOH, H$_2$O, 70%; (d) Al/Hg,THF, H$_2$O, 65%; (e) (i) Ac$_2$O; (ii) porcine pancreatic lipase, 35-45%; (f) PPh$_3$, DEAD, THF, 60%; (g) **143**, BF$_3$*Et$_2$O, THF, 20%; (h) Dowex-50W, MeOH, 95%; (j) *t*BuOOH, VO(acac)$_2$, benzene, 67%; (k) BzONa, H$_2$O, 80%; (l) Ac$_2$O, pyridine, 82%; (m) Tf$_2$O, DMAP, CH$_2$Cl$_2$, 61%; (m) K$_2$CO$_3$, MeOH, 72% .

Scheme 17. Hudlický`s synthesis of *ent*-7-deoxypancratistatin.

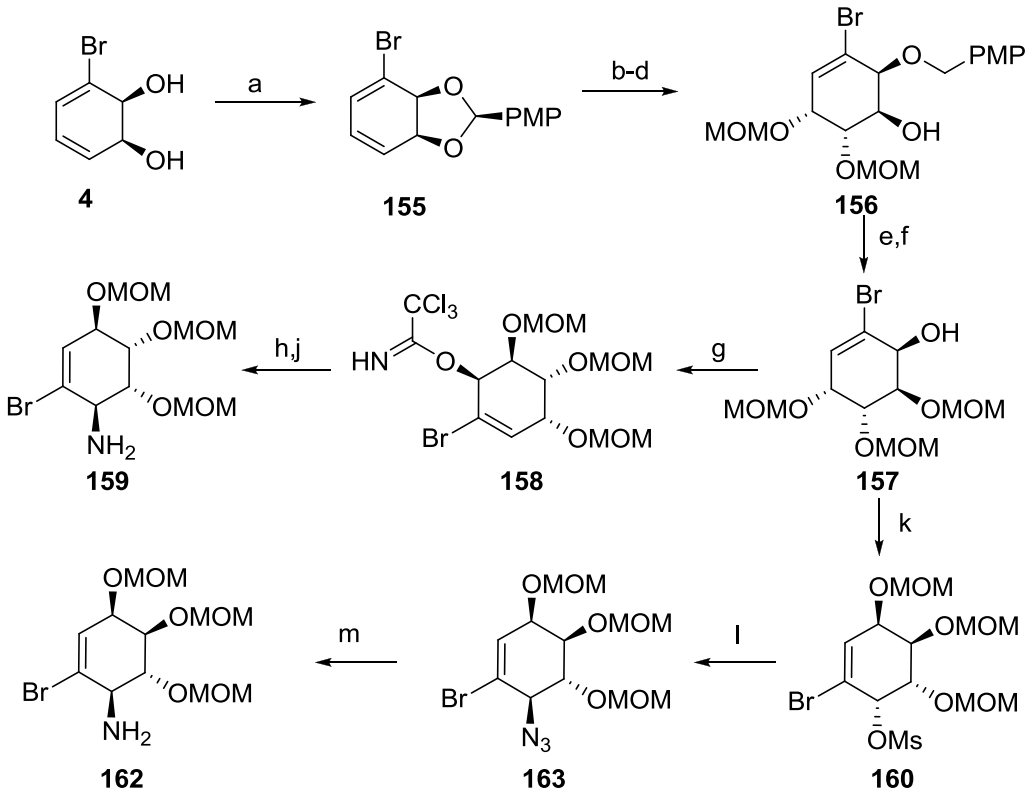
DeShong[73] developed a palladium-catalyzed racemic approach towards pancratistatin-type compounds. It consists in coupling between aryl fragments and the Pd π-allyl complex formed from allyl carbonate of type **152**, Scheme 18. The synthesis began with a hetero-Diels Alder reaction between cyclohexadiene **150** and an acylnitroso compound formed *in situ* to provide bicyclic oxazine **151**, which was reduced with molybdenum hexacarbonyl and protected with ethyl chloroformate to give allylic alcohol **152**. This compound underwent Hiyama coupling with silane **98** to furnish intermediate **46** and its regioisomer (not shown) in a 1:1.6 ratio. After isolation the desired isomer was submitted to a Bischler-Napieralski cyclization followed by installation of the *trans*-diol moiety by treatment peacid epoxidation and ring opening to yield compound **154**.



Reaction conditions: (a) $HONHCO_2Et$, nBu_4IO_4, $CHCl_3$, DMF, 71%; (b) $Mo(CO)_6$, $MeCN/H_2O$, 62%; (c) $ClCO_2Et$, pyridine, CH_2Cl_2, 83%; (d) **98**, $Pd(dba)_2$, TBAF, THF, 81%; (e) $POCl_3$, P_2O_5, $(Me_3Si)_2O$, 56%; (f) (i) H_2O_2, HCOOH, (ii) NaOH, H_2O, 51%.

Scheme 18. DeShong`s synthesis of 3,4,7-dideoxypancratistatin.

Banwell[74, 75] used chiral diol **4** to secure access to a few unnatural enantiomers of narciclasine-type compounds. The strategy utilised for the formation of the core of the compound was similar to that used by Hudlický[25, 26] and relies on tandem Suzuki coupling and lactam formation between arylboronic ester **165** and different chiral vinyl bromides to provide access to narciclasine type compounds in a short sequence, Scheme 19. Diol **4** was protected as *p*-methoxybenzyl acetal **155**, which was selectively dihydroxylated. Protection of the diol with methoxymethyl groups and reductive ring opening of the PMB-acetal gave vinylbromide **156**. Further manipulation of protective groups secured access to the key intermediate **157**. Subjection of this alcohol to Overmann rearrangement conditions produced amine **159,** which served as building block for *ent*-narciclasine (*ent*-**1**), Scheme 20. Mesylation of alcohol **157** followed by azidation and hydrolytic Staudinger reaction provided building block **162** for synthesis of *ent*-3-*epi*-lycoricidine (**167**), Figure 12.

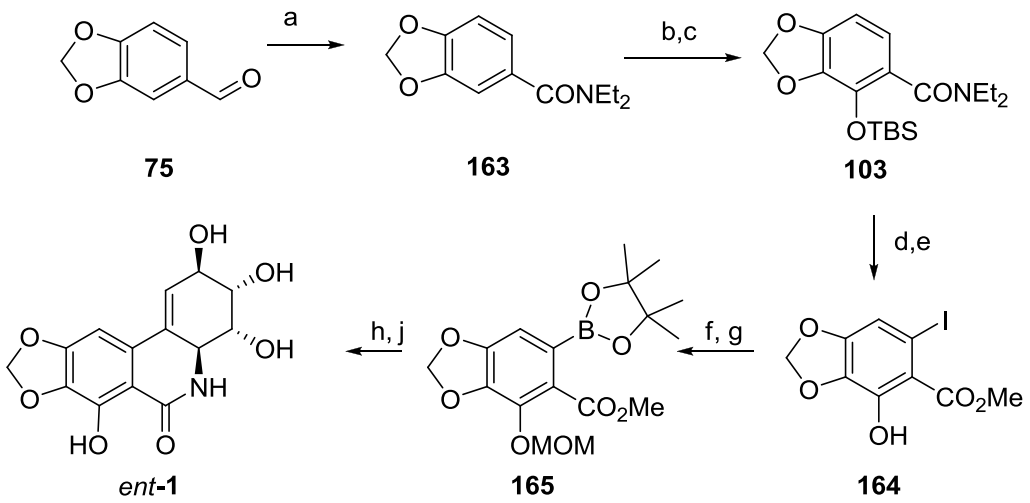


Reaction conditions: (a) *p*MBDMA, TsOH, CH_2Cl_2; (b) OsO_4, NMO, acetone, H_2O, 65% for 2 steps; (c) MOMCl, NaH, DIPEA, THF, 75%; (d) DIBAL, $PhCH_3$, 84%; (e) MOMCl, NaH, Et_3N, THF, 90%; (f) DDQ, CH_2Cl_2, H_2O, 95%; (g) Cl_3CCN, DBU, CH_2Cl_2, 3Å sieves; (h) K_2CO_3, *o*DCB, μwave, 78% for 2 steps ; (j) DIBAL, $PhCH_3$, 89%; (k) MsCl, DMAP, Et_3N, CH_2Cl_2; (l) NaN_3, DMF, 95% for 2 steps; (m) PPh_3, H_2O, THF, 99%.

Scheme 19. Banwell`s approach to a common building block in the synthesis of *ent*-analogues.

The aromatic building block **165** for coupling was produced in seven steps starting from piperonal (**75**), which was efficiently transformed to diethylamide **103** *via* Corey–Gilman–Ganem oxidation, Scheme 20. It was subjected to a typical metallation/borylation/oxidation sequence and protection conditions, further ortho-metallation, halogenation and deprotection with Meerwein`s reagent provided phenol ester **164**. After protection with methoxymethyl group it was borylated with

pinacolborane in presence of a palladium catalyst. Boronate **165** was submitted to an one-pot Suzuki coupling and amide formation to form the skeleton of the final compound, which upon acidic deprotection provided *ent*-narciclasine (**1**). A similar strategy was applied to the synthesis of several other analogues, Figure 12.



Reaction conditions: (a) NaCN, MnO_2, Et_2NH, 58%; (b) (i) *s*BuLi, TMEDA, THF; (ii) $B(OMe)_3$; (iii) AcOH, H_2O_2, 90%; (c) TBSCl, imidazole, CH_2Cl_2, 94%; (d) (i) *s*BuLi, TMEDA, THF; (ii) I_2, 90%; (e) Me_3OBF_4, Na_2HPO_4, MeCN, 62%; (f) MOMCl, NaH, THF, 99%; (g) pinacolborane, $Pd(OAc)_2$, CyJohnphos, MeCN, 54%; (h) **159**, K_2CO_3; $PhCH_3$, H_2O, $Pd(PPh_3)_4$, μwave, 63%; (j) TMSBr, CH_2Cl_2, 48%.

Scheme 20. Banwell`s synthesis of *ent*-narciclasine.

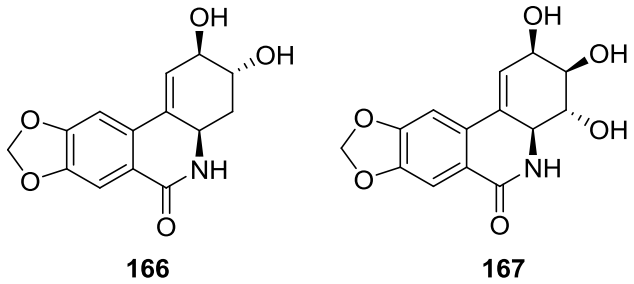
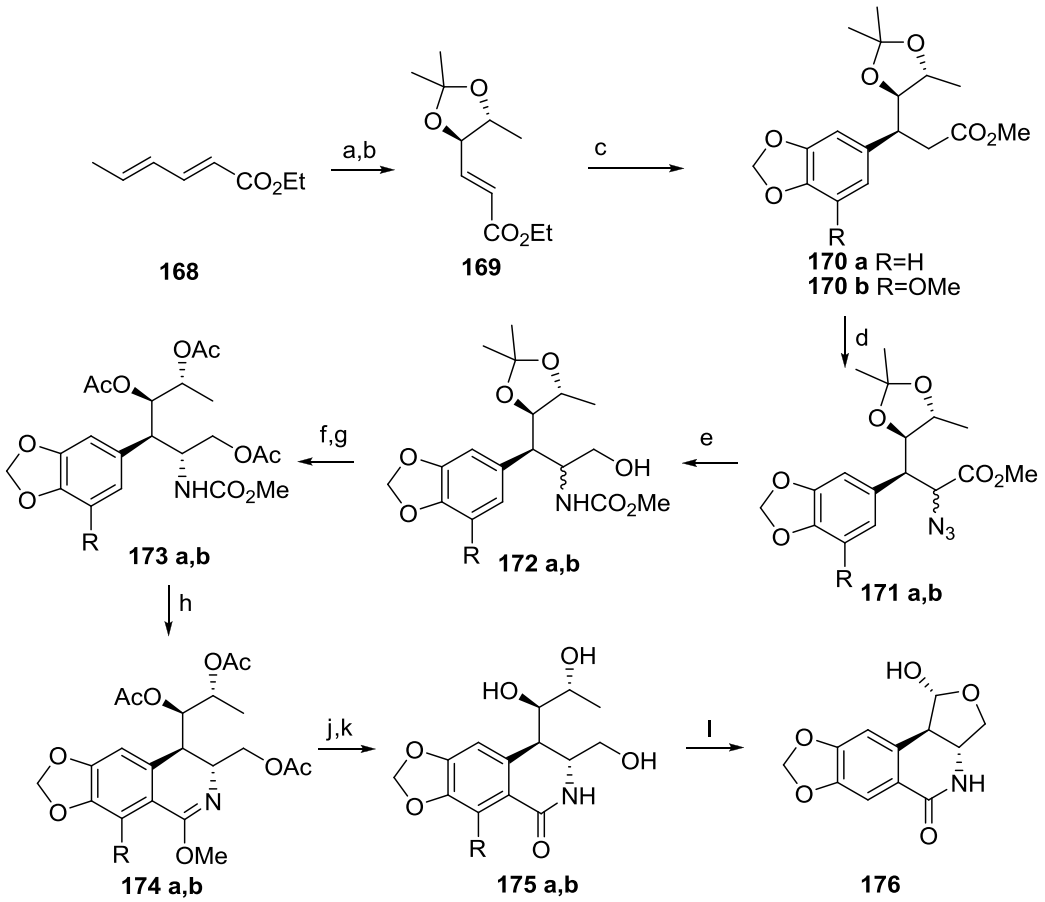


Figure 12. Different analogues of *ent*-narciclasine.

Kornienko[76] designed a synthesis of truncated analogues that lacked the cyclohexane structure of the C-ring. This approach began with Sharpless asymmetric dihydroxylation

of ethyl sorbate **168** followed by acetonide protection to produce a chiral acrylic acid ester **169**, Scheme 21. Stereoselective conjugate addition of aryl cuprates led to compounds **170a** and **170b**, enolates of which were subjected to azidation reaction, which unfortunately led to a mixture of diastereomers. After reduction of the mixture of azides **171** with lithium aluminium hydride and protection as carbamate, the required trans-isomer of **172** was purified. Standard reprotection with acetates (before the key Bischler-Napieralski cyclization) led to triacetate **173**. The procedure for the cyclization, originally designed by Banwell,[64] was modified and studied in some detail. Changing the base to 2-chloropyridine led to a clean conversion to imidate **174**. Subsequent demethylation with trimethyliodosilane and basic hydrolysis provided compounds **175a** and **175b -** open C-ring analogues of **11** and **2** respectively. 7-Deoxy analogue **175a** was carried further to periodate assisted cleavage of diol to produce truncated cyclic analogue **176**.



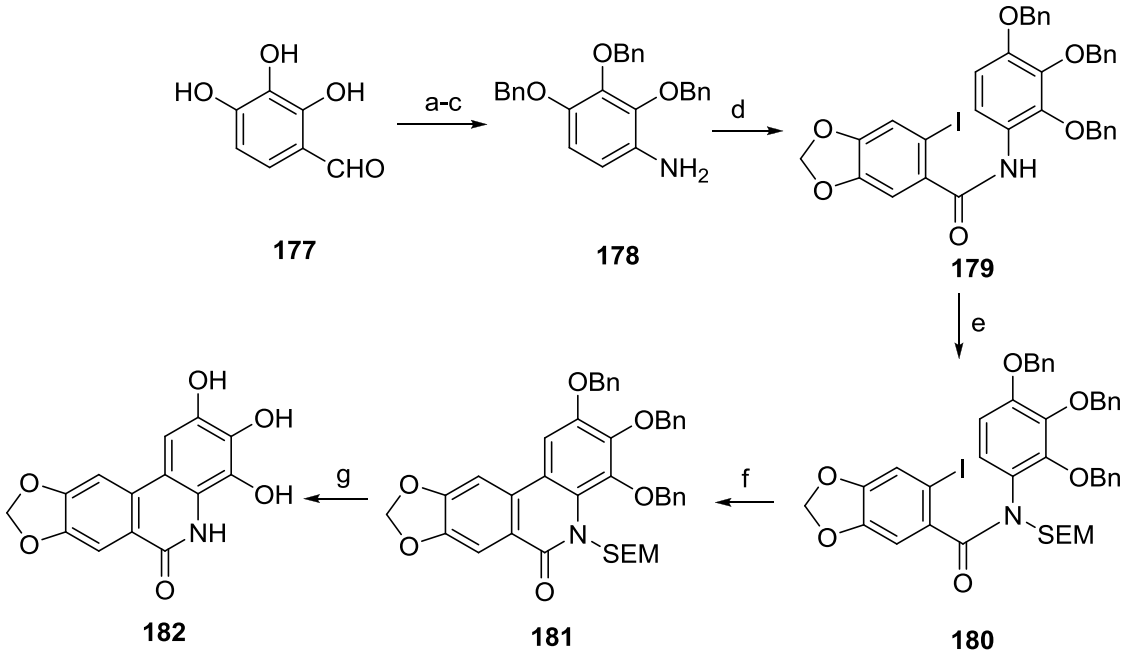
168 **169** **170 a** R=H **170 b** R=OMe **171 a,b** **172 a,b** **173 a,b** **174 a,b** **175 a,b** **176**

Reaction conditions: (a) AD-mix-β, *t*-BuOH, 78%; (b) DMP, *p*-TsOH, 94%; (c) ArMgCuBr, TMSCl, 84 % for **171a**, 74% for **171b** ; (d) LDA, trisyl N_3, 77%; (e) (i) $LiAlH_4$; (ii) MeOCOCl, 86% a, 65% b, (f) Dowex MeOH, 56% a, 50% b ; (g) AcOH, 88% a, 96% b; (h) Tf_2O, 2-ClPy, 71% a, 59% b; (j) TMSI, MeCN, 84% a, 95% b; (k) K_2CO_3, MeOH, 71% a 83% b; (l) $NaIO_4$, MeOH, 82%.

Scheme 21. Kornienko`s synthesis of truncated C-ring analogues of 7-deoxypancratistatin.

Kim[77] has been studying the effect of complete aromatisation of C-ring on the biological activity of narciclasine and pancratistatin. Because of the lack of any chiral centers it was possible to develop a short scalable route towards these compounds. Synthesis began with complete benzylation of 2,3,4-trihydroxybenzaldehyde **179**, followed by Pinnick oxidation of the intermediate to the carboxylic acid, which underwent a Curtius rearrangement to aniline **178**, Scheme 22. Coupling of this product with acyl chloride

made *in situ* from 6-iodopiperonylic acid (**56**) provided amide **179**. In order to undergo intramolecular coupling the secondary amide **179** needed to be transformed to the tertiary amide **180**, which was achieved by alkylation of **179** with SEMCl. Direct arylation was achieved in high yield in the presence of palladium acetate, *tris*(*o*-tolyl)phosphine and silver salt as a base. One-pot global deprotection of the coupled product **181** was achieved by hydrogenation and treatment with trifluoroacetic acid to furnish the final trihydroxy product **182** in low yield. Kim also described an analogous synthesis of three aromatic dihydroxy analogues (not shown), neither of these componds displayed anticancer activity.

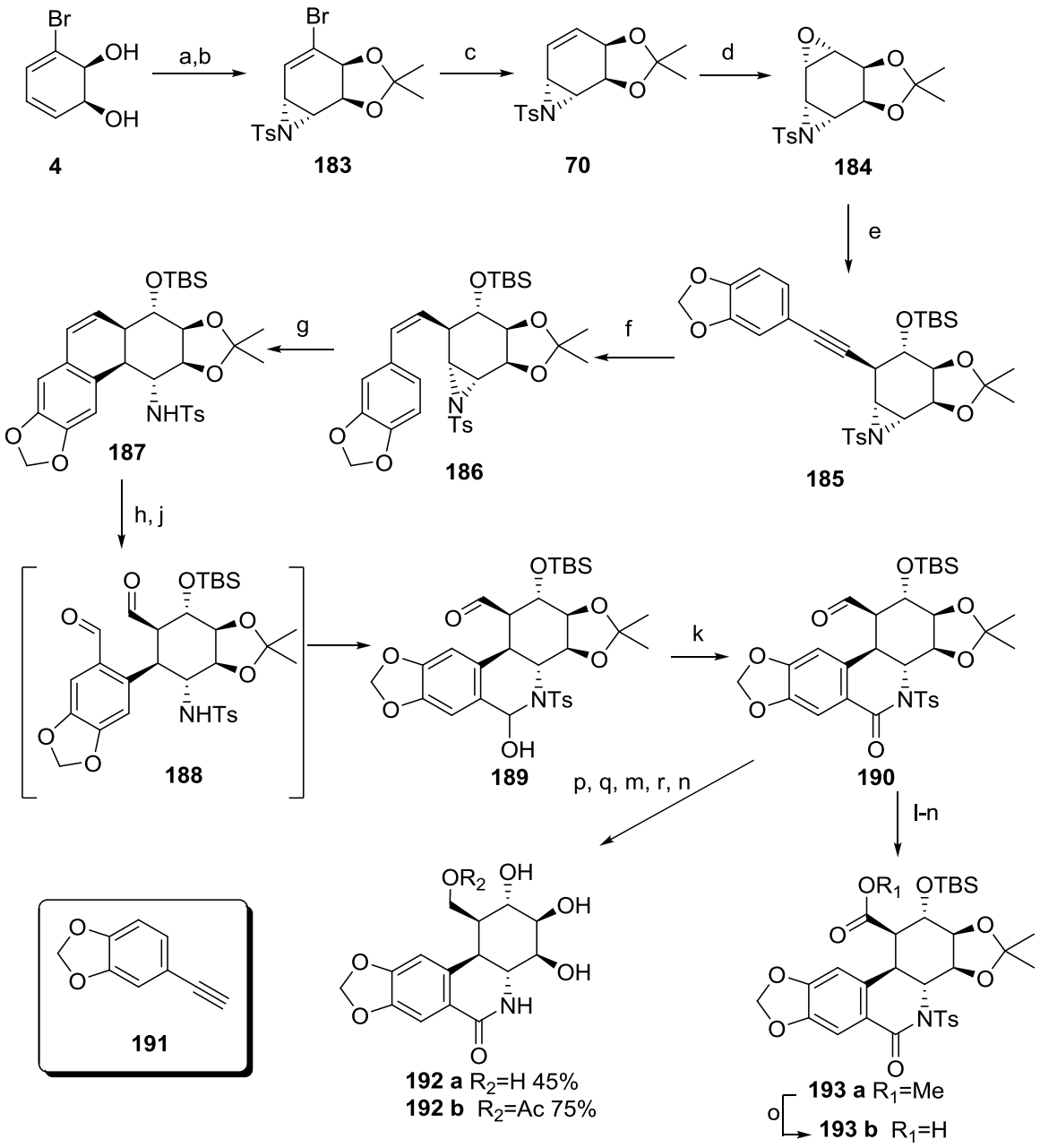


Reaction conditions: (a) BnBr, K_2CO_3, KI, DMF; (b) $NaClO_2$, NaH_2PO_4, MeCN, *t*-BuOH, H_2O; (c) (i) DPPA, Et_3N; (ii) KOH, H_2O, 84% for two steps; (d) **56**, $(COCl)_2$, benzene, DMF, 64%; (e) SEMCl, NaH, DMF, 77%; (f) $Pd(OAc)_2$, $P(o\text{-}Tol)_3$, Ag_2CO_3, 95%; (g) (i) $H_2/Pd(OH)_2$, EtOAc; (ii)TFA, CH_2Cl_2, 18%.

Scheme 22. Kim`s approach to C-aromatic analogues.

Hudlický[78, 79] designed a synthetic pathway towards a variety of compounds, which are homologues of 7-deoxypancratistatin at position C-1. The syntheses originated from

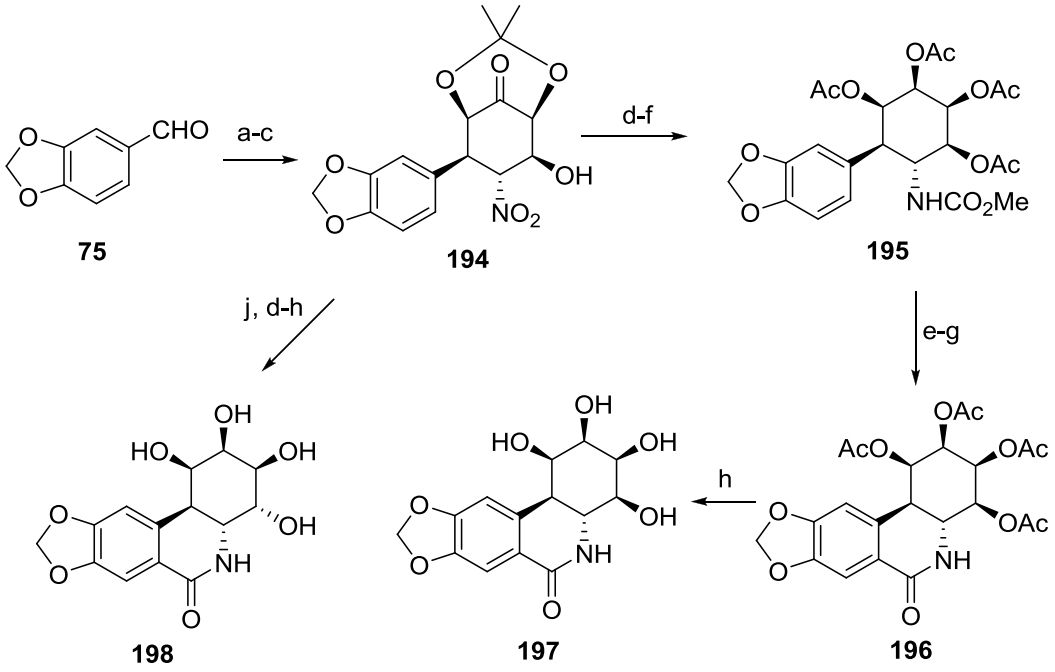
chiral diol **4**, which upon protection was immediately submitted to an aziridination reaction following the procedure developed by Yamada[80] and Evans[81] to provide aziridine **183** in moderate yield, Scheme 23. Radical dehalogenation in the presence of tributyltin hydride led to vinylaziridine **70**, which upon epoxidation with *m*-chloroperbenzoic acid in refluxing 1,2-dichloroethane yielded a mixture of diastereomeric epoxides in a 3:1 ratio (major diastereomer **184** shown). After fractional recrystallization, the major isomer was submitted to epoxide opening reaction with the alkynylalane, formed from alkyne **191**.The product of epoxide opening was protected with *tert*-butyldimethyl triflate without isolation to provide **185** in good yield. Selective reduction of the alkyne moiety to the *cis*-alkene was achieved upon treatment with borane and after isolation, alkene **186** was submitted to solid phase silica gel-catalyzed aziridine opening.[82, 83] The product of this opening, namely phenantrene **187**, was isolated in moderate yield and submitted to oxidative cleavage in order to produce dialdehyde **188**. This transformation was achieved by sequential treatment with osmium tetroxide, reduction of the ketoalcohol with sodium borohydride, and cleavage of diol with sodium periodate. Transient dialdehyde **188** was not isolated but instead was allowed to undergo the cyclization to hemiaminal **189**. The next step involved the oxidation of the hemiaminal to amide **190** in order to furnish the complete skeleton of the final product. This intermediate **190** was used as a point of divergence to produce, *via* oxidation and deprotection, carboxylic acid **193a** and methyl ester **193b**. Reduction of aldehyde **181** with sodium borohydride and acetylation led to alcohol **192b**, and acetate **192a** respectively, following deprotection.



Reaction conditions: (a) 2,2-DMP, TsOH; (b) PhI=NTs, $Cu(acac)_2$, MeCN, 52% for two steps; (c) *n*Bu_3SnH, THF, 70%; (d) *m*CPBA,1,2-DCE, 96%; (e) (i) **191**, *n*BuLi, Me_2AlCl, toluene; (ii) TBSOTf, Et_3N, CH_2Cl_2, 77%; (f) BH_3, cyclohexene, THF, 76%; (g) SiO_2, 120°C, 52%; (h) OsO_4, NMO, CH_2Cl_2, 89%; (j) (i) $NaBH_4$, dioxane, EtOH; (ii) $NaIO_4$, dioxane, H_2O, 82%; (k) IBX, DMF, 61%; (l) (i) *m*CPBA, NaH_2PO_4, H_2O; (ii) CH_2N_2, Et_2O, 83%; (m) Na/naphthalene, DME, 58%; (n) HCl, MeOH, 69%; (o) LiOH, MeOH, 95%; (p) $NaBH_4$, EtOH, 85%; (q) Ac_2O, DMAP, CH_2Cl_2, 81%; (r) TBAF, THF, 74% for two steps.

Scheme 23. Hudlický`s approach towards C-1 homologues of pancratistatin.

Alonso[84, 85] developed yet another general strategy towards different analogues of pancratistatin and applied it towards the synthesis of 2-*epi*-7-deoxypancratistatin (**197**) and 2,4-di*epi*-7-deoxypancratistatin (**198**), Scheme 24. The strategy was identical to the one described previously for pancratistatin, (see Figure 10). The [3+3] annulation was performed with a derivative of piperonal (**75**), and dihydroxyacetone (**83**) to produce protected cyclohexanone **194**. A divergence point between synthesis of pancratistatin and its analogues lies in the nature of the stereoselective reduction of the carbonyl function in **194**. Because of steric hindrance from the acetonide protecting group the reduction gave the opposite stereoselectivity than that observed with a similar reduction,[53] which led to the synthesis of the natural product. Reduction of the nitro group, deprotection and reprotection afforded intermediate **195**, which was submitted to cyclization under the standard Banwell procedure[64] followed by deprotection to provide to 2-*epi*-7-deoxypancratistatin **197** in racemic form. A similar strategy was applied to the synthesis of racemic 2,4-di*epi*-7-deoxypancratistatin (**190**): intermediate **194** was submitted to a base-induced epimerization of the C-4 free hydroxyl group, followed by the same reduction-deprotection-cyclization sequence as described above. These compounds can also be prepared in an enantiopure form by utilisation of previously developed enantioselective protocol.[53]

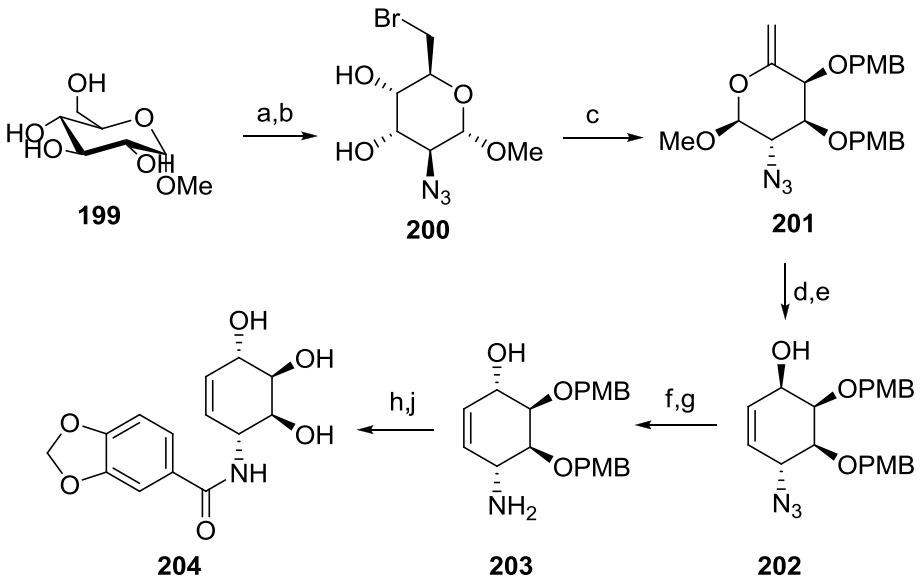


Reaction conditions: (a) $HO(CH_2)_2NO_2$, NH_4OAc, AcOH, 43%; (b) IBX, EtOAc, 73%; (c) **83**, DMF 43%; (d) (i) $NiCl_2$, $NaBH_4$, MeOH; (ii) 2,2-DMP, TsOH; (e) $ClCO_2Me$, DMAP, CH_2Cl_2, 49% for 2 steps; (f) (i) MeOH, TsOH, CH_2Cl_2; (ii) Ac_2O, pyridine, 74%; (g) Tf_2O, DMAP, CH_2Cl_2, 48% (h) K_2CO_3, MeOH, 84%; (j) KOH, THF, 30%.

Scheme 24. Alonso`s synthesis of *epi* 7-deoxypancratistatin analogues.

2.1.3.2. B-ring analogues

Chapleur[86] was one of the first to systematically study the pharmacophore of pancratistatin. He utilized the chiral pool approach towards open B-ring analogues or *seco*-analogues. Methyl D-glucopiranoside (**199**) was subjected to protection, azidation and regioselective oxidative opening to produce bromide **200**, Scheme 25. Reprotection and sequential elimination led to intermediate **201**. The key step of this sequence was the transformation of pyranoside **201** *via* Ferrier rearrangement to provide cyclohexane core, which upon Luche reduction provided azido alcohol **202**.



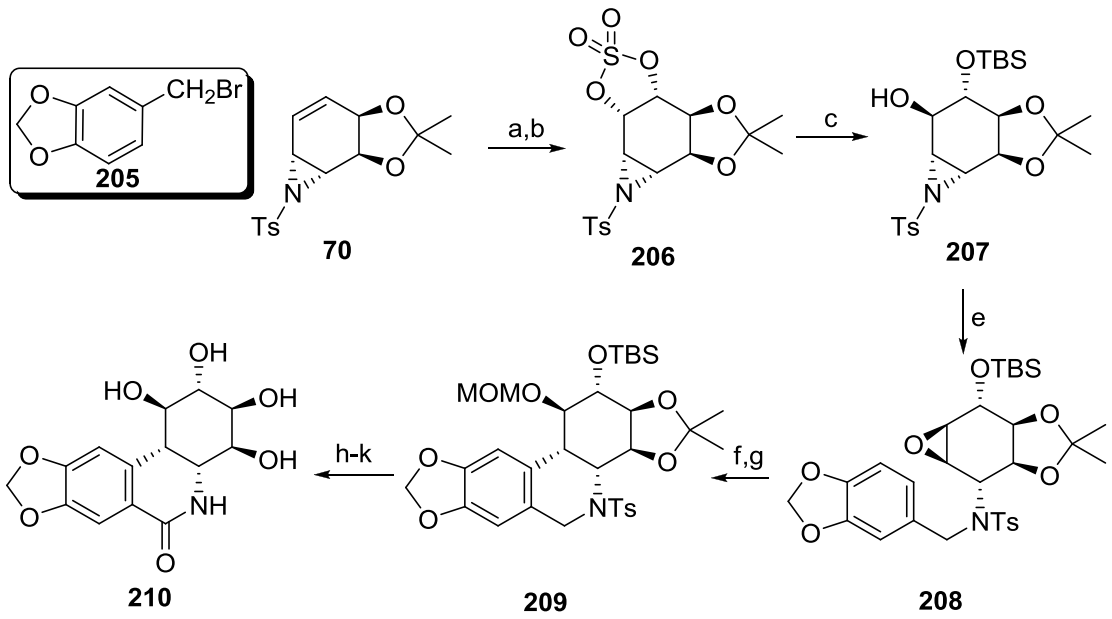
Reaction conditions: (a) (i) PhCHO, $ZnCl_2$, 75%; (ii) TsCl, DMAP, pyridine, 70%; (iii) MeONa, MeOH, $CHCl_3$ 70%; (b) (i) NaN_3, NH_4Cl, H_2O, monoglyme, 95%; (ii) NBS, $CaCO_3$, CCl_4; (iii)NaOMe, MeOH, 60%; (c) PMBBr, NaH, DMF, 70%; (d) $Hg(TFA)_2$, acetone, H_2O, 55%; (e) $NaBH_4$, $CeCl_3*7H_2O$, MeOH, THF, 76%; (f) $PhCO_2H$, PPh_3, DEAD, THF, 83%; (g) $LiAlH_4$, Et_2O, 78%; (h) **102**, BOP, Et_3N, THF, 90%; (j) DDQ, CH_2Cl_2, H_2O, 61%.

Scheme 25. Chapleur`s synthesis of open B-ring analogues.

In order to establish the correct stereochemical relation of hydroxyl groups in **202**, the C-2 hydroxyl (pancratistatin numbering) was subjected to a Mitsunobu reaction, followed by the reduction of azide, which led to the protected conduramine **203**, which was coupled to piperonylic acid (**102**) and deprotected to provide open-chain analogue **204**. This compound was shown to be completely inactive.

Hudlický[87] synthesisized the previously unknown 10-*epi*-7-deoxypancratistatin (**210**) in order to compare the effect of *cis*-junction of the B-ring on anticancer activity, Scheme 26. Synthesis started from the common intermediate aziridine **70**, used before in few total syntheses of Amaryllidaceae alkaloids. It was transformed to cyclic sulfate **206** which was opened with ammonium benzoate, to provide upon protection and hydrolysis *trans*-

diol **207**. Formation of the lithium salt of this compound was followed by immediate aza-Payne rearrangement and, after alkylation with piperonyl bromide (**205**), provided epoxide **208**. Intramolecular opening of the epoxide catalyzed by Lewis acid provided the skeleton of the final compound **209** with the required *cis*-stereochemistry. Finally oxidation of the benzylic position and deprotection provided 10-*epi*-7-deoxypancrastatin (**210**). Anticancer assays of this analogue showed comlete lack of activity/

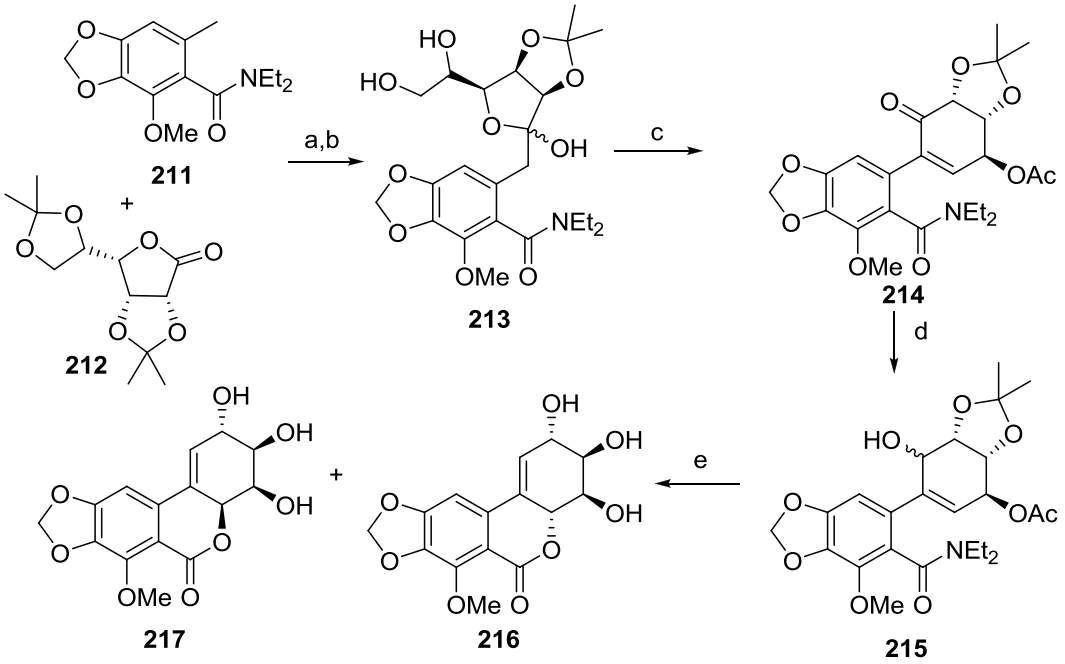


Reaction conditions: (a) $RuCl_3{*}H_2O$, $NaIO_4$, H_2O, EtOAc, 85%; (b) SO_2Cl_2, Et_3N, CH_2Cl_2, 93%; (c) (i) $PhCO_2NH_4$, DMF; (ii) THF, H_2SO_4, H_2O, 90%; (d) (i) TBSCl, imidazole, DMF; (ii) NaOMe, THF, 63%; (e) (i) *t*BuLi, THF; (ii) **205**, nBu_4NI, 68%; (f) Me_2AlCl, CH_2Cl_2, 68%; (g) MOMCl, DIPEA, 97%; (h) $RuCl_3{*}H_2O$, $NaIO_4$, H_2O, CCl_4, CH_3CN, 50%; (j) Na/naphtalene, DME, 75%; (k) HCl, MeOH, 68%.

Scheme 26. Hudlický`s synthesis of 10-*epi*-7-deoxypancrastatin.

Chapleur[88] studied the effect of replacement of nitrogen in the lactam moiety by oxygen on biological activity. Ortho-metallated amide **211** was reacted with protected D-glucuronolactone (**212**) followed by selective acid-catalyzed acetonide deprotection led to polyol **213**, which was oxidised with sodium periodate and was subjected to DBU-

induced Knoevenagel condensation and acetate protection, Scheme 27. The resulting enone **214** was subjected to Luche reduction, which unfortunately did not appear to be stereoselective. Removal of acetate and exposure to acid conditions led to a smooth formation of the lactone and deprotection of acetonide group. Two lactones, **216** and **217**, were separated and subjected to biological testing. Although this is one the shortest syntheses of the pancratistatin analogues, it did not lead to lactone **216** stereoselectively, and both of these compounds showed lack of any anticancer activity.

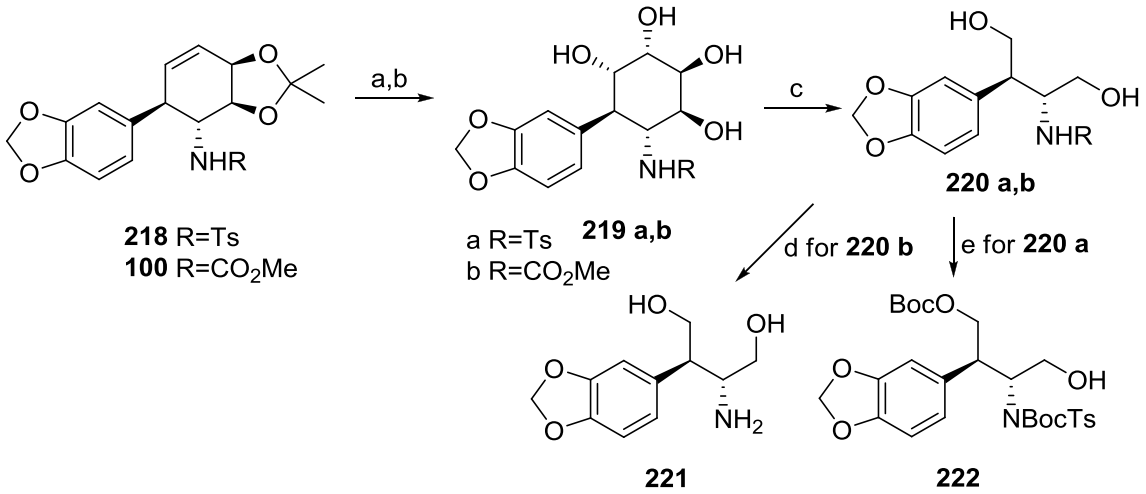


Reaction and conditions: (a) *s*-BuLi, THF, 70%; (b) AcOH, H_2O, 90%; (c) (i) $NaIO_4$, MeOH; (ii) Na_2CO_3, DBU, THF; (iii) Ac_2O, pyridine, 70%; (d) $NaBH_4$, $CeCl_3$*$7H_2O$, 90%; (e) (i) NaOMe, MeOH; (ii) AcOH:H_2O (9:1), TFA, 67%.

Scheme 27. Chapleur`s synthesis of lactone analogues.

Hudlický used the strategy previously developed in his group for the synthesis of 7-deoxypancratistatin (**11**)[56] for the synthesis of analogues with open C and B-rings.[26]The key step was oxidation of intermediate tetraols **219** in order to produce open ring

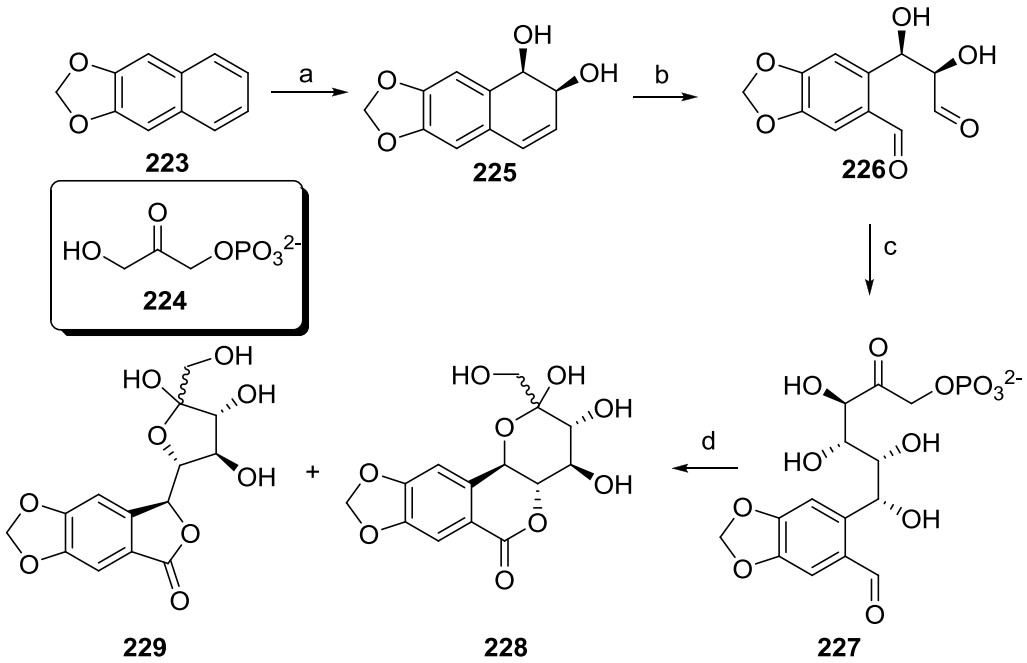
analogues of **11**, Scheme 28. Oxidative cleavage was followed by reduction to produce diols **220**, then depending on a protecting compound was either deprotected completely to provide an amine **221** or protected with *t*-butoxycarbonyl group to produce **222**. Both of these compounds were subjected to biological studies, which showed that only **222** was slightly active, section 2.1.4.



Reaction conditions: (a) (i) $RuCl_3$, $NaIO_4$, EtOAc,MeCN, 75% a, 69% b ; (b) TFA, THF H_2O; (c) (i) $NaIO_4$, acetone, H_2O ; (ii) $NaBH_4$, MeOH, 60% a 45% b; (d) (i) KOH, MeOH; (ii) HCl, MeOH, 82% (e) NaH, Boc_2O, THF, 85% .

Scheme 28. Hudlický`s synthesis of truncated ring-opened analogues.

Fessner[89] used a biocatalytical approach towards synthesis of sugar-based heterocyclic analogue **228**, Scheme 29. He utilised whole-cell oxidation of naphtalene (**223**) with recombinant *E.Coli* JM109 (pDTG121) as a first step. The product of oxidation, diol **225**, was isolated as a single enantiomer in good yield and subjected to ozonolysis. Dialdehyde **226** was, without isolation, carried through the sequential enzymatic aldol reaction with dihydroxyacetone phosphate **224**, dephosphorylation, and oxidation to produce a mixture of desired pyranoside **228** and the by-product furanoside **229** in 4 steps. Unfortunately, despite enzyme screening, no selective way to form **228** were found.

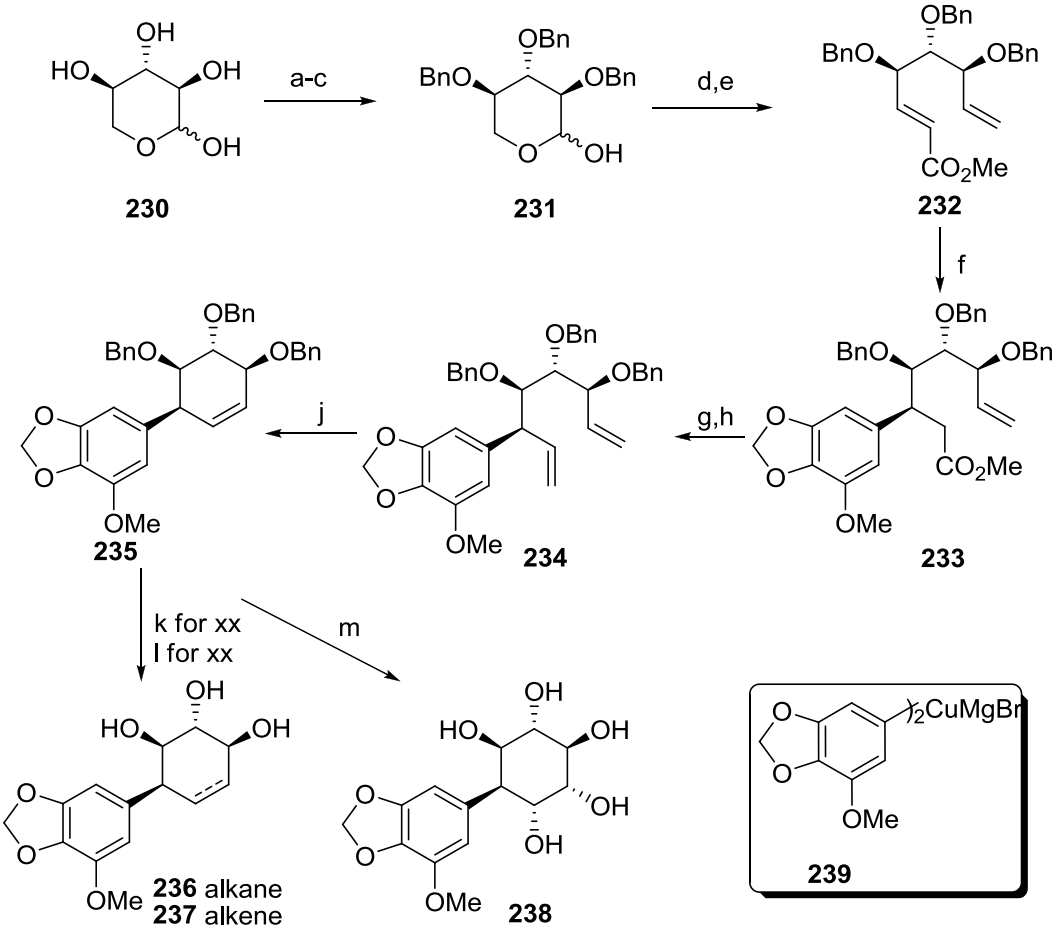


Reaction conditions: (a) JM109 (pDTG121), 64%; (b) (i) O_3, MeOH; (ii) Me_2S; (c) rhamsulose 1-phosphate aldolase, **224**; (d) (i) acid phosphatase; (ii) Br_2, $BaCO_3$, 10% (1:1 mixture **228**:**229**) for 3 steps .

Scheme 29. Fessner`s biocatalytic approach towards analogues of 7-deoxypancratistatin.

Complementary to the efforts of Chapleur, Kornienko[90, 91] described an approach to the analogues of pancratistatin that completely lack the amide moiety but retain stereochemical relation between A- and C-ring. The synthesis started by selective benzylation of D-xylose **230** to a protected hemiacetal **231**, which was submitted to a Wittig reaction, and oxidation followed by the second Wittig reaction, Scheme 30. Conjugate addition of aryl cuprate **239** to the acrylic ester **232** led to the stereoselective formation (ratio >50:1) of product **233**. In order to cyclize C-ring of this compound, the ester moiety was reduced and transformed to the alkene by means of Grieco elimination.[92] Ring-closing methathesis of two terminal alkenes was achieved by 1st generation Grubbs catalyst (**240**) and led to the general precursor **235**, which can be selectively transformed to alkene analogue **237**, alkane **236**, or diol **238** depending on

conditions. By varying arylcuprates different analogues were obtained and all were assayed for anticancer activity with complete lack of any activity.

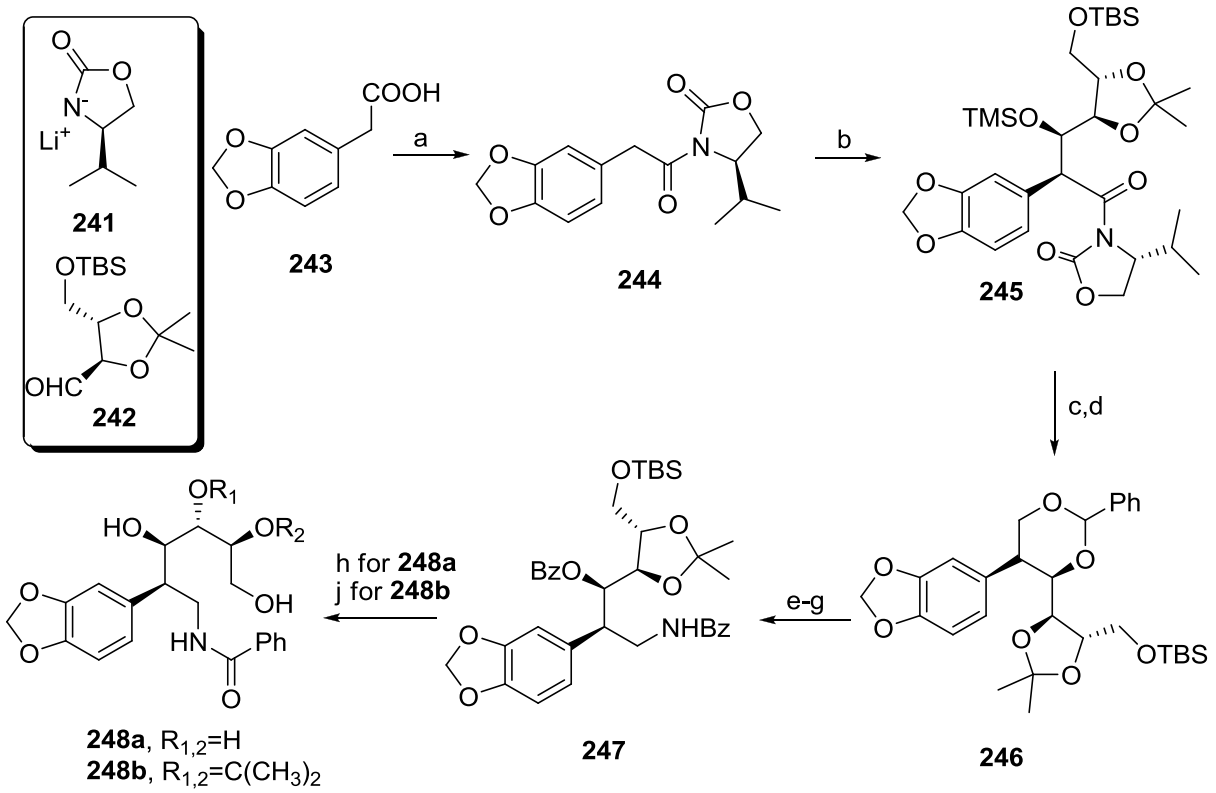


Reaction conditions: (a) MeOH, $SOCl_2$; (b) BnCl, TBAI, NaH, DMF; (c) H_2SO_4, dioxane, H_2O, 70% for 3 steps; (d) $Ph_3P=CH_2$, THF; (e) (i) $(COCl)_2$, DMSO, Et_3N; (ii) $Ph_3P=CHCO_2Me$, CH_2Cl_2, 84% for two steps; (f) **239**, TMSCl, 97%; (g) $LiAlH_4$, Et_2O; (h) (i)*o*-$NO_2PhSeCN$, Bu3P; (ii) H_2O_2, 84%; (j) $(Cy_3P)_2(PhCH=)RuCl_2$ (**240**), CH_2Cl_2, 96%; (k) Li/NH_3, THF, 61%; (l) H_2, Pd/C, MeOH, 95%; (m) (i)OsO_4, NMO, acetone; (ii) H_2, Pd/C, MeOH, 84%.

Scheme 30. Kornienko`s approach to an open B-ring analogues.

Further studies towards refining of pharmacophore were performed by McNulty.[93] The key step of his approach consisted of Evans aldol reaction, which allowed the establishment, in one step, of the correct stereochemistry at C10b and C1 carbons. Synthesis started by the installation of Evans auxiliary onto phenylacetic acid **243**,

Scheme 31. Product of this reaction **244** was submitted to aldol reaction in presence of magnesium chloride with chiral aldehyde **242**, produced from tartaric acid, to provide product **245** with 95:5 selectivity. Reductive removal of the auxiliary group and reprotection with benzylidene acetal led to acetal **246**. Regiolselective oxidative cleavage of the acetal with *N*-bromosuccinimide provided a convenient leaving group which was replaced by azide and followed by reduction and concomitant O to N benzoyl migration, thus ensuring the installation of the amide fragment in product **247**. Two different deprotection condtions led to product **248a** and **248b** respectively. These open-chaing analogues displayed no anticancer activity.



Reaction conditions: (a)(i) PivCl, Et_3N, Et_2O; (ii) **241**, THF, 84%; (b) **242**, $MgCl_2$, Et_3N, TMCl, EtOAc. 98%; (c) $LiBH_4$, THF, MeOH, 88%; (d) $PhCH(OMe)_2$,TsOH, CH_2Cl_2, 91%; (e)NBS, AINM, benzene, 70%; (f) NaN_3, DMF, 85%; (g) H_2, Pd/C, THF, 94%; (h) HCl, MeOH, 87%; (j) TBAF, THF, 96%.

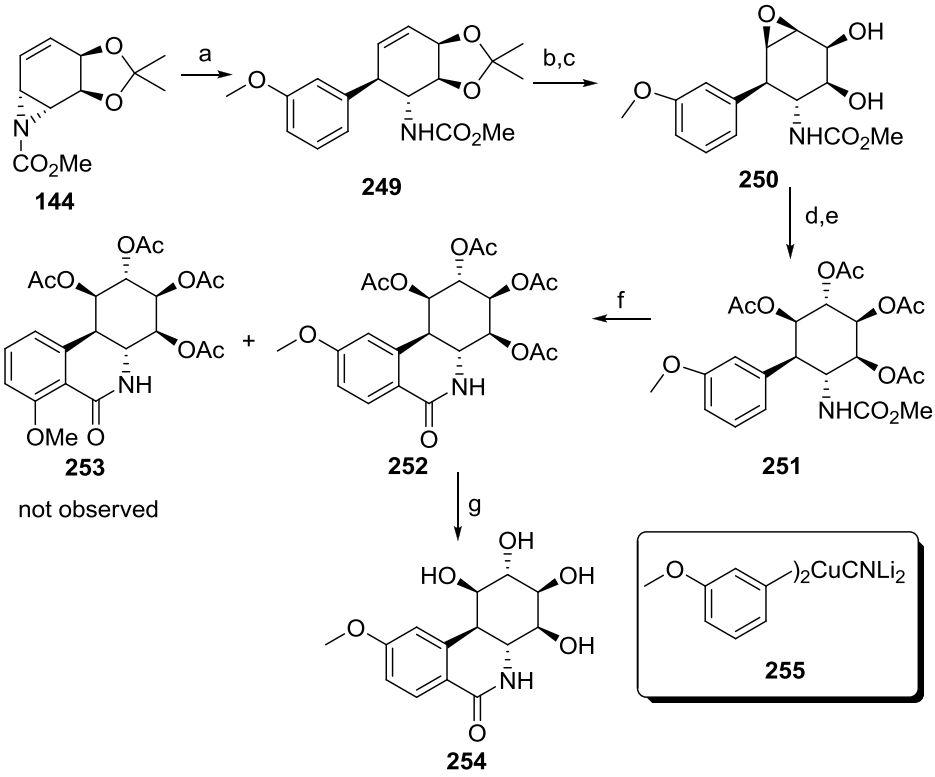
Scheme 31. McNulty`s synthesis of truncated B- and C–ring analogues.

2.1.3.3. A-ring analogues

Analogues of A-ring modifications comprise the most underrepresented group of the analogues of isocarbostyril Amaryllidaceae congeners. The main reason is that most synthetic efforts were focused on solving the complexity of B- and C-rings therefore less challenging A-ring modifications received little attention.

Only a few compounds are known that represent A-ring modification of pancratistatin. One of the first examples is the 7,8-dideoxypancratistatin (**254**) synthesized by Hudlický,[72] Scheme 32. General strategy was similar to the synthesis of actual **4**,[56] but

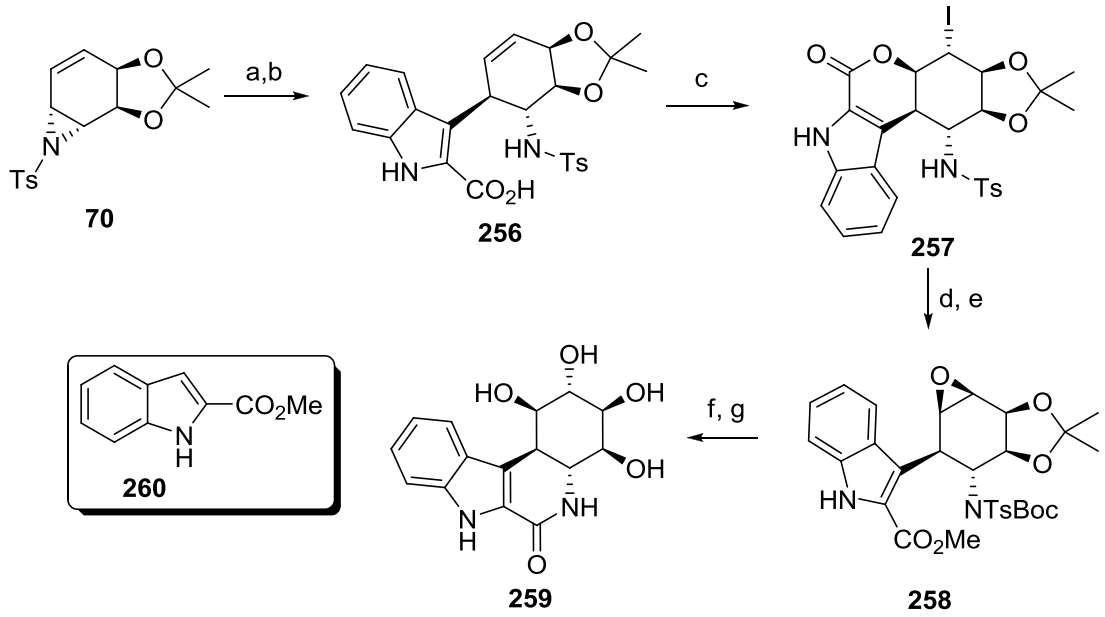
different aryl cuprate **255** was used. Bischler-Napieralski cyclization of tetraacetate **251** would expect to form of two regioisomers **252** and **253**. But in fact only one isomer , namely **252**, was formed in the course of reaction and after deprotection it afforded **254**. Activity of this compound was shown to be two orders of magnitude lower than pancratistatin.



Scheme 32. Hudlický`s synthesis of 7,8-dideoxypancratistatin.

The effect of complete removal of methylenedioxy fragment from A-ring was later studied by the same group.[82] It was reasoned that indole moiety has similar steric and to some extent electronic properties of original arylmethylenedioxy fragment. In order to produce such analogue **259** vinylaziridine **70** was subjected to silica gel-catalyzed

nucleophilic opening by methyl 2-indolecarboxylate (**260**) to produce cyclohexene **256**, Scheme 33. Iodolactonization of this alkene followed by basic hydrolysis with lithium methoxide led to formation of epoxide. In order to remove the tosyl protecting group amide was transformed into *t*-butoxycarbamate **258** and submitted to one-electron reduction to remove tosyl group, followed by prolonged exposure to wet silica-gel upon heating furnished the final indole β-carboline **249**. Indole substitution rendered compound completely inactive.

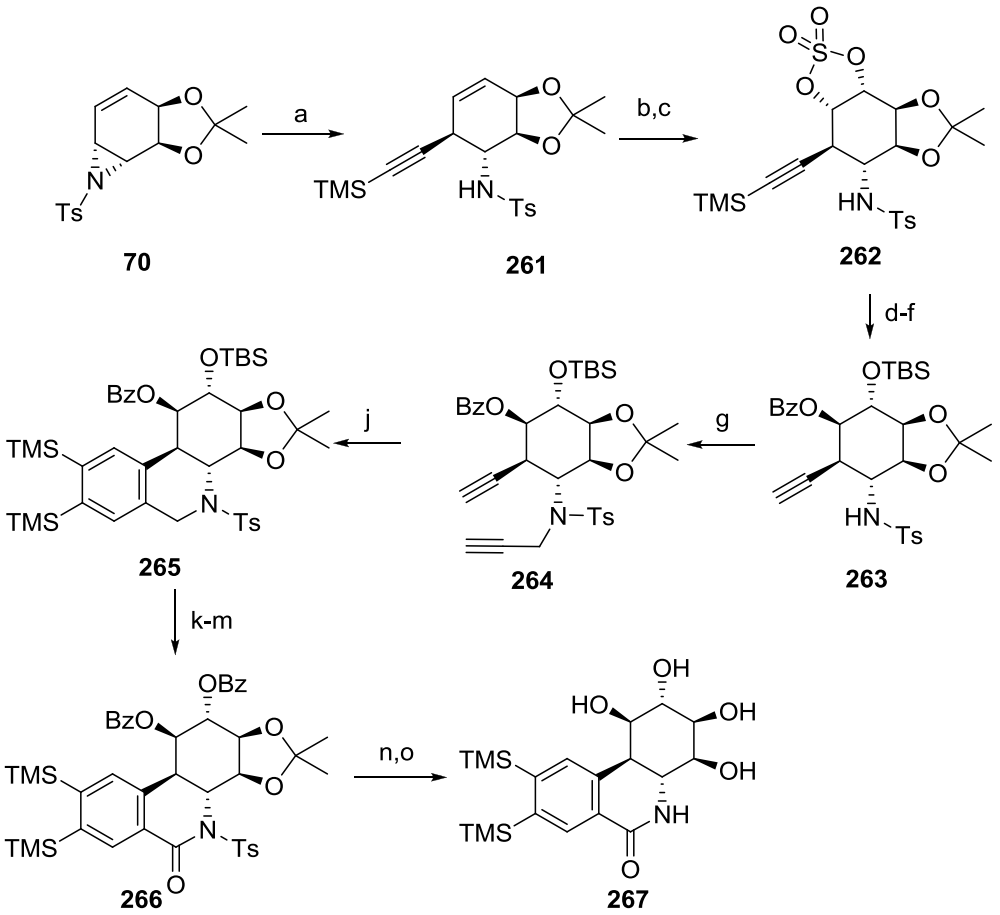


Reactions and conditons: (a) **260**, SiO_2, 68%; (b) LiOH, H_2O, 95%; (c) I_2, $NaHCO_3$, THF, 71%; (d) LiOMe, MeOH, 85%; (e) Boc_2O, 88%; (f) Na, naphthalene, DME; (g) SiO_2, H_2O, 31%.

Scheme 33. Hudlický`s synthesis of indole analogue of pancratistatin.

Further development of modification of A-ring was based on a [2+2+2] cycloaddition strategy.[94,95] This time the effect of replacement of methylenedioxy moiety with silyl groups was studied. Synthesis was based on nucleophilic opening of vinylaziridine **70**

with alane generated from TMS acetylene (**268**), Scheme 34. The resulting enyne **261** was then selectively dihydroxylated with osmium tetroxide and transformed to a cyclic sulfate **262**. Nucleophilic opening of sulfate with ammonium benzoate established the required *trans*-diol stereochemistry followed by selective desilylation of terminal alkyne and alcohol protection to provide **263**. Alkylation of amide moiety with propargyl bromide led to the key dialkyne **264**, which was submitted to [2+2+2] cycloaddition with bis(TMS)acetylene (**269**) catalyzed by cyclopentadienyl cobalt dicarbonyl. This reaction furnished skeleton of the desired product **265** in good yield.



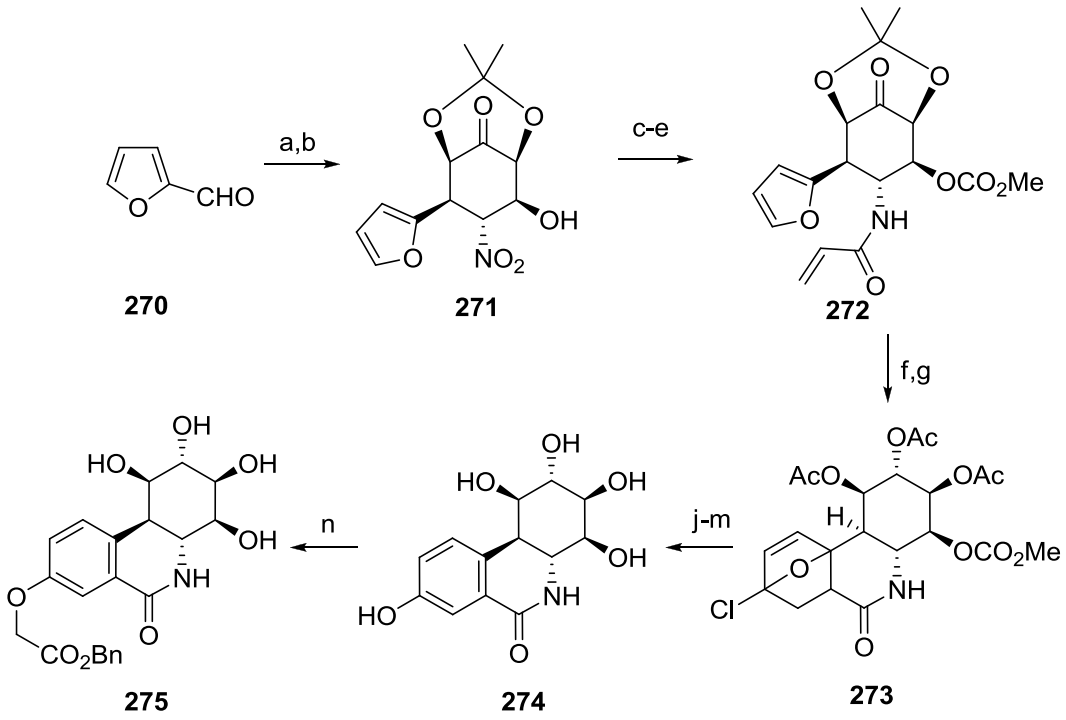
Reactions conditons: (a) (i) TMS-acetylene (**268**) , *n*BuLi, $AlCl_3$; (ii) 2,2-DMP, acetone , TsOH; 69%; (b) OsO_4, NMO, CH_2Cl_2, 44%, (76% brsm); (c) SO_2Cl_2, Et_3N, CH_2Cl_2 82%; (d) (i) $PhCO_2NH_4$, DMF, (ii) H_2SO_4, H_2O, 33%; (e) TBAT, MeCN, 84%; (f) TBSCl, imidazole, DMF, 89%; (g) propargyl bromide, NaHMDS, $(nBu)_4NI$, 79%; (j) BTMSA (**269**), $CpCo(CO)_2$, xylene, 83%; (k) TBAF, THF, 84%; (l) PhCOCl, pyridine, 86%; (m) $NaIO_4$, $RuCl_3$, Na_2CO_3, CCl_4, MeCN, H_2O, 33%; (n) (i) Na/napthalene, THF; (ii) NaOMe, MeOH; 32%; (o) Dowex 50WX-100, MeOH, 94%.

Scheme 34. Hudlický`s synthesis of disilyl pancratistatin analogue.

In order to oxidise the benzylic position by high-valent ruthenium reagents, protecting group exchange was performed and, after oxidation, phenanthridone **266** was obtained. Detosylation and sequiential basic and acidic hydrolysis led to bis-trimethylsilyl-7-deoxypancratistatin **267**, completely inactive in anticancer assays.

Alonso[85] developed general organocatalytic [3+3] route towards synthesis of A-ring analogues. The early stages of synthesis followed his general approach towards

nitrocyclohexanones such as **271** from furfural (**270**), Scheme 35. Protection and reduction of nitro group in **271** was followed by acylation with acryloyl chloride in order to furnish starting material **272** for the intramolecular Diels-Alder reaction. Chlorination of furan with *N*-chlorosuccinimide helped to facilitate formation of Diels-Alder product **273** as well as subsequent aromatisation. Deprotection of the acetonide was followed by reduction at C-2 to ensure correct relative stereochemistry, and further reprotection with acetates. Finally, aromatisation was achieved by treatment with sodium methoxide and led simultaneously to the complete deprotection of the ester groups. Analogue **274,** with a free phenolic group was subsequently transformed to protected version **275** and both of these compound were submitted to anticancer testing and showed no activity.



Reactions and conditons: (a) (i) $HO(CH_2)_2NO_2$, KOH; (ii) IBX, EtOAc; (b) (i) **83**, pyrrolidine, DMF; (ii) PPTS, CH_2Cl_2, 55% for 2 steps; (c) 2-methoxypropene, PPTS, 97%; (d) Ni/Raney, MeOH, 86%; (e) (i) acryloyl chloride, Et_3N, DMAP; (ii) PPTS, MeOH; (iii) $ClCO_2Me$, DMAP, 72%; (f) NCS, DMF, 95%; (g) toluene, $NaHCO_3$, 53%; (j) Dowex50WX, MeOH; (k) $NaBH(OAc)_3$, 1,2-DCE, THF; (l) Ac_2O, DMAP, Et_3N, 85% for 3 steps; (m) NaOMe, MeOH, 93%; (n) benzyl bromoacetate, K_2CO_3, nBu$_4$NI, DMF, 25%.

Scheme 35. Alonso`s synthesis of 7,9-dideoxypancratistatin analogues.

2.1.3.4. Semi-synthesis from natural products.

Because of the relatively high abundance of narciclasine in common perennials of temperate climate such as *Narcissus pseudonarcissus* and *Narcissus poeticus*[10] in comparison with other congeners, it has always been considered an attractive route to utilize **1** as a starting material for synthesis of analogues. One of the first effort in this field was reported by Mondon and Krohn,[7] who studied hydrogenation and other transformations of narciclasine to different derivatives. Synthesis of these compounds and biological studies performed on them constited one of the first examples of refining the

pharmacophore of Amaryllidaceae congeners. Several of the compounds produced in this study showed activity comparable with narciclasine itself in assays against cancer cell lines, especially trans-dihydronarciclasine (**9**), other derivatives such as *cis*-dihydronarciclasine (**276**), *iso*-narciclasine (**277**), and 7-methylnarciclasine (**278**), showed significantly reduced activity in comparison with natural compounds, Figure 13.

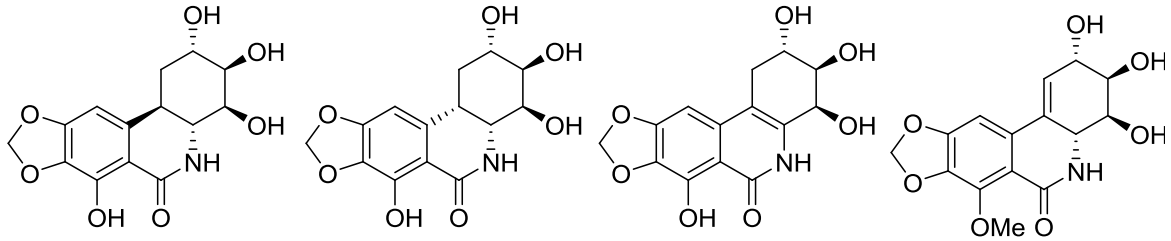
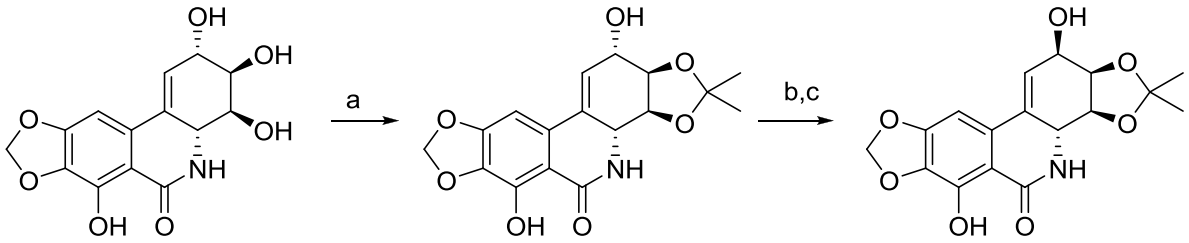


Figure 13. First synthetic derivatives of narciclasine.

During their studies Mondon and Krohn first observed selective protection of the *syn*-diol to its ketal to form compounds of type **279**, Scheme 36. Selective oxidation of the allylic alcohol in position C-2 lead to unstable ketone (not shown) which upon reduction provided mixture of protected 2-*epi*-narciclasine **280** and **279**.

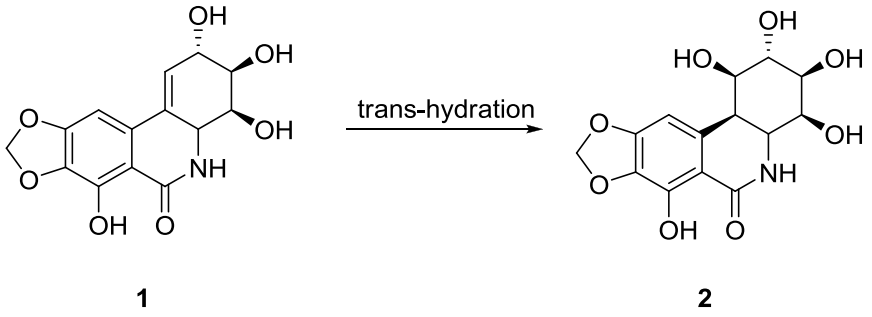


Reaction conditions: (a) acetone, $HC(OEt)_3$, TsOH, 74%; (b) MnO_2, THF, 87%; (c) $NaBH_4$, EtOH.

Scheme 36. Synthesis of protected 2-*epi*-narciclasine.

Further development in the area of hydrogenation of narciclasine and 7-deoxynarciclasine was later provided by Pettit[96, 97] in order to improve the selectivity of hydrogenation towards the most active congener, namely *trans*-dihydronarciclasine (**12)**. Despite screening of different catalysts and condtions,[97] the best selectivity was obtained with palladium on carbon, but is still remains low (**9:276:277**=51:47:2) on milligram scale. Upon scaling the reaction, the ratio of **12**:**276** drops to 30:62.

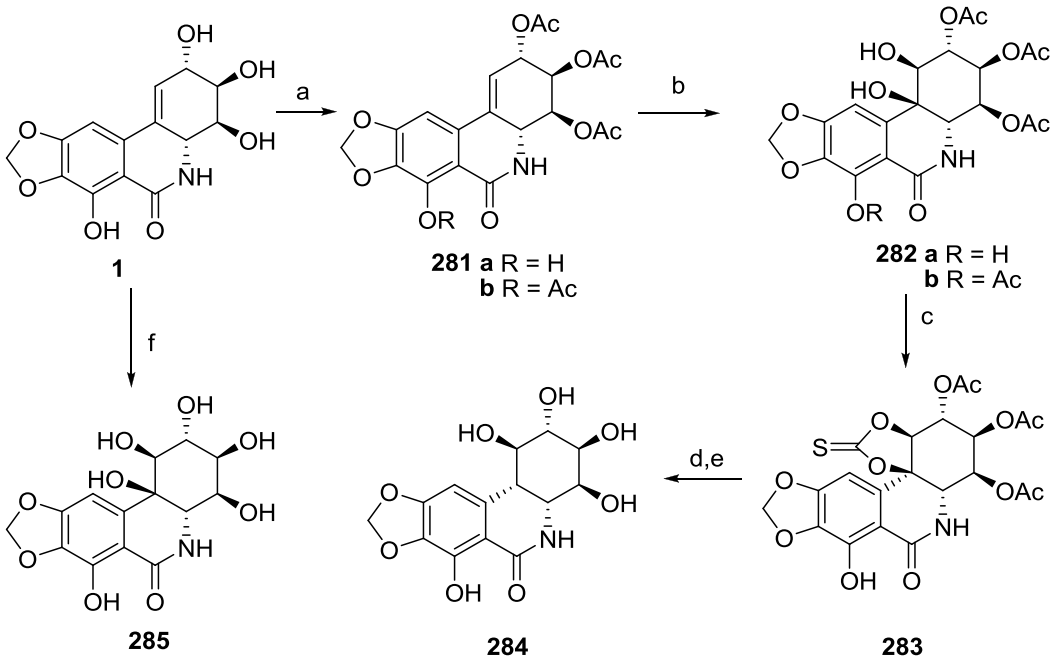
Systematic studies on the conversion of narciclasine to different analogues were performed by Pettit.[9, 48, 97-102] His major goal was to convert narciclasine (**1**) to pancratistatin (**2**), since his group has been largely involved in the discovery and the modification of pancratistatin skeleton. This task seems to be trivial, as the only difference is the presence of a hydroxyl group at C-1 and the absence of a double bond. In theory, this transformation can be achieved by stereoselective hydration of narciclasine, Figure 14.



1 2

Figure 14. Theoretical transformation of narciclasine to pancratistatin.

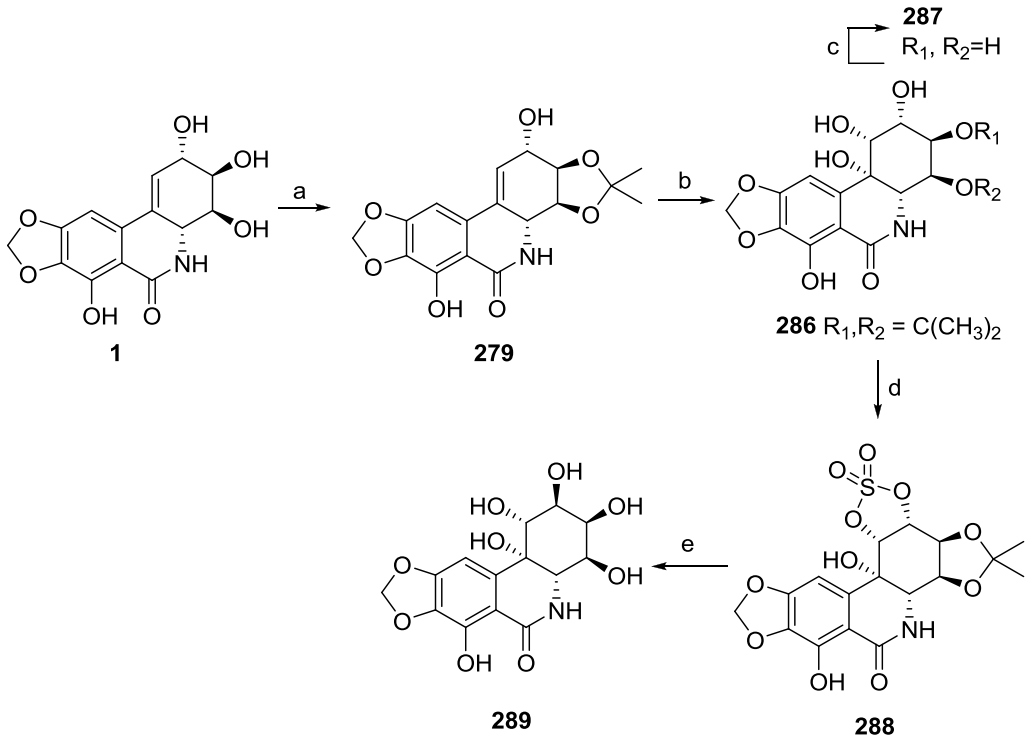
In order to study this transformation Pettit performed studies on face-selective dihydroxylation of narciclasine. Depending on protection groups pattern, selectivity between α- and β-face dihydroxylation can be achieved. His studies showed[102] that unprotected narciclasine (**1**) as well as acetate protected **281a** upon different dihydroxylation conditions, including Upjohn and Sharpless asymmetric procedures, provided selective β-face dihydroxylation and yielded 10b-(*R*)-hydroxypancratistatin (**285**), Scheme 37. Different attempts to reduce the benzylic hydroxyl upon hydrogenolysis conditions in order to produce **2** did not provide the desired product. Later Pettit[98] studied radical approaches to reduce thiocarbonate derivative **283** but instead 10b-(*S*) *epi*-pancratistatin (**284**) was produced.



(a) Ac_2O, pyridine, 29% for **281a**, 80% for **281b**; (b) OsO_4, NMO, $(DHQD)_2PHAL$,DMF, H_2O, 85%; (c) TCDI, 2-butanone, 72%; (d) Bu_3SnH, AIBN, PhMe, 50%; (e) K_2CO_3, MeOH, H_2O, 64%, (f) OsO_4, NMO, hydroquinone, DMF, H_2O, 72%,

Scheme 37. Selective dihydroxylation and synthesis of 10b-(S) *epi*-pancratistatin.

Introduction of a more sterically demanding protecting group, such as an acetonide, can alter the selectivity of dihydroxylation preferentially to α-face. Pettit[101] showed that upon exposure of narciclasine acetonide **279** to Sharpless asymmetric dihydroxylation conditions led to product **286** and its β-diastereomer in the ratio of 2:1, Scheme 38. Deprotection of the major product under acidic conditions led to another hydroxylated analogue -10b-(*S*)-1-*epi*-pancratistatin (**287**). In order to achieve the inversion of the C-1 position in this structure the cyclic sulfate **288** was formed by treatment of **286** with thionyl chloride and subsequent oxidation. Unfortunately, nucleophilic opening of the cyclic sulfate **288** with cesium benzoate at position C-2, afforded only 10b-(*S*)-1-*epi*-2-*epi*-pancratistatin (**289**).

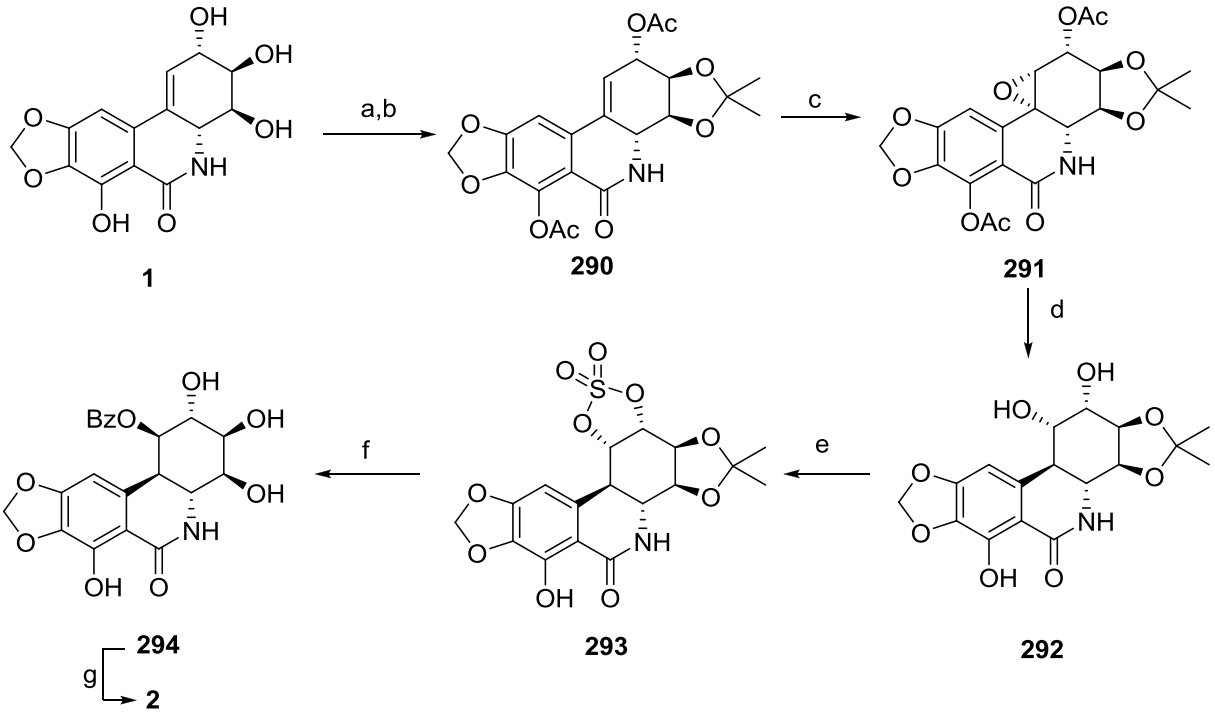


Reaction and conditions: (a) 2,2-DMP, DMF, TsOH, 97%; (b) OsO_4, NMO, $(DHQD)_2PHAL$,DMF, H_2O 54%; (c) H_2SO_4, THF, H_2O, 52%; (d) (i)$SOCl_2$, Et_3N, THF; (ii) $RuCl_3*3H_2O$, $NaIO_4$, $MeCN/CCl_4/H_2O$; 63%; (e) (i) $PhCO_2H$, Cs_2CO_3, DMF; (ii) H_2SO_4, THF, H_2O; (iii) K_2CO_3, MeOH, 33%.

Scheme 38. Selective α-dihydroxylation of narciclasine.

In 2001 Pettit developed[48] a relay synthesis of pancratistatin (**2**) form narciclasine (**1**). The first steps of which consisted in stepwise protection of narciclasine by acetonide and acetates to form the protected intermediate **290**, Scheme 39. Protected narciclasine was submitted to the epoxidation with *m*CPBA in phosphate buffer. Face selective epoxidation led to epoxide **291**, which was in turn submitted to hydrogenation on Pd/C. Unfortunately, this step turned to be a bottleneck of the synthesis and provided a mixture of products, amongst which the desired diol **292** was produced in only 28% yield. This diol was transformed to a cyclic sulfate **293**, and then was followed by nucleophilic opening with cesium benzoate and acid deprotection to produce C-1 benzoate of pancratistatin **294**. Upon basic hydrolysis the ester was hydrolyzed to produce

pancratistatin (**2**) in 10 steps from narciclasine (**1**). Despite quite a low yield this study provided essential data about biological activity, since all of the intermediates were subjected to anticancer cell assays. C-1 benzoate ester (**294**) in particular has shown the highest activity of all known analogues and congeners of *Amaryllidaceae* alkaloids, see 2.1.4.2.

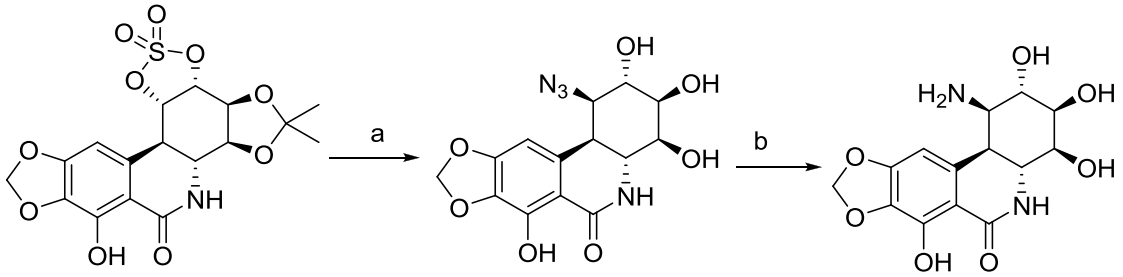


Reaction conditions: (a) 2,2-DMP, DMF, TsOH, 97%; (b) Ac_2O, pyridine, 81%; (c) *m*CPBA, NaH_2PO_4, CH_2Cl_2, H_2O, 52%; (d) H_2, Pd/C; EtOAc, 28%; (e) (i) $SOCl_2$,Et_3N, THF; (ii) $RuCl_3$, , $NaIO_4$, MeCN, H_2O, CCl_4, 45%; (f) (i) $PhCO_2H$, Cs_2CO_3, DMF; (ii) H_2SO_4, H_2O, THF, 74%; (g) K_2CO_3, MeOH, 75%.

Scheme 39. Relay synthesis of pancratistatin form narciclasine.

Later the same strategy of nucleophilic opening of sulfate **293** was utilised by Marion from Pierre Fabre Laboratories[103] to generate a library of nitrogenated derivatives of pancratistatin. Cyclic sulfate **291** was produced the way described by Pettit,[48] and was subjected to nucleophilic opening by sodium azide, and deprotected to provide 1-azido-

pancratistatin **295** which can, in turn, be reduced to amine **296**, Scheme 40. Library of different amides and amines, Figure 15, was generated from **296**. Some of these compounds have shown significant biological activity exceeding that of narciclasine itself. Most pronounced activity was shown by compounds with flat lypophylic substituents at position C-1, such as benzamide, see 2.1.4.2.



Reaction conditions: (a) (i) NaN_3, DMF, 80°C; (ii) H_2SO_4, THF, 72% for 2 steps; (b) Pd/C, H_2, 52%;

Scheme 40. Amino C-1 analogues.

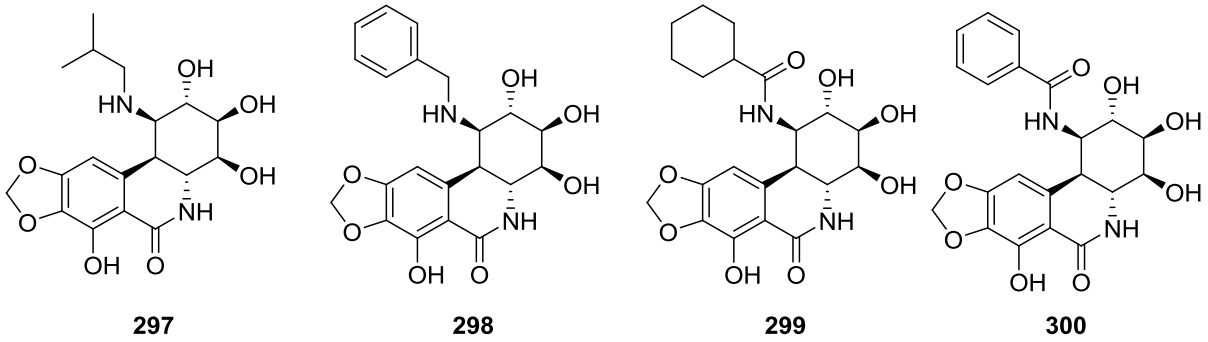
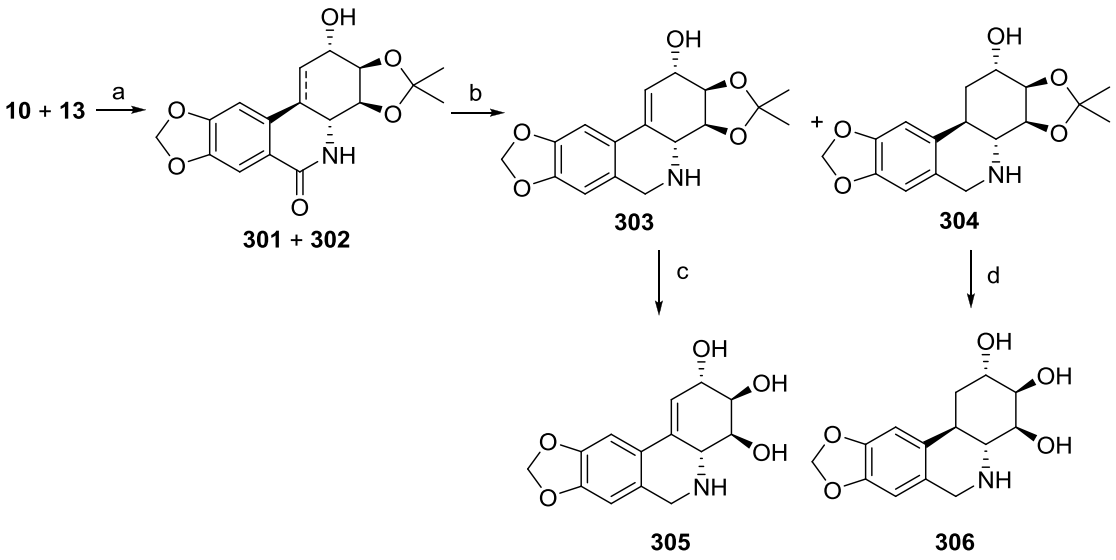


Figure 15. Library of amino C-1 derivatives.

Synthesis of two more analogues stemmed from a study on the separation of lycoricidine (**10**) and *trans*-dihydrolycoricidine (**13**) from bulbs of *P. Littorale* by Pettit.[99] Protection of the chromatographically inseparable mixture of these compounds as acetonides led to the mixture of **301** and **302**, Scheme 41. Further protection with *t*-butyldimethylsilyl

group and reduction of amide moiety with lithium alumohydride produced separable mixture of amines **303** and **304**. Upon deprotection, the two new analogues without amide moiety **305** and **306** were isolated as their respective hydrochloride salts and subjected to biological studies.



Reaction and conditions: (a) 2,2-DMP, *p*-TsOH,DMF; (b) (i) TBSCl, imidazole, DMF; (ii) $LiAlH_4$, Et_2O; TBAF, THF; 53% **303**, 18% **304**; (c) (i) H_2SO_4, H_2O, THF, CH_2Cl_2; (ii) $NaHCO_3$; (iii) HCl, MeOH, 68%; HCl, MeOH, 63% .

Scheme 41. Pettit`s synthesis of amine analogues.

Kiss[104] extensively studied various structural modifications of narciclasine (**1**) to produce series of 28 diverse analogues. Some of the compounds which have shown anticancer activity are presented on Figure 16. He utilized a previously described strategy in order to generate selectively protected C-2 and C-7 substituted esters **307-309**, 4-substituted amides **310**, and 2-*epi*-amines **311**, **312**. Stability toward hydrolysis as well as anticancer activity of all of these derivatives were studied.

All of the studies presented above show the wealth of methods developed toward the modification and synthesis of different analogues of Amaryllidaceae congeners. Some of

these syntheses demonstrated to be very efficient in terms of the step count and yield, but synthetic efficiency cannot be a single criterion for the estimation of success in the field of synthesis of bioactive molecules. Therefore the next part will be dedicated to discussion of biological activity of produced analogues as well as possible ventures in this field.

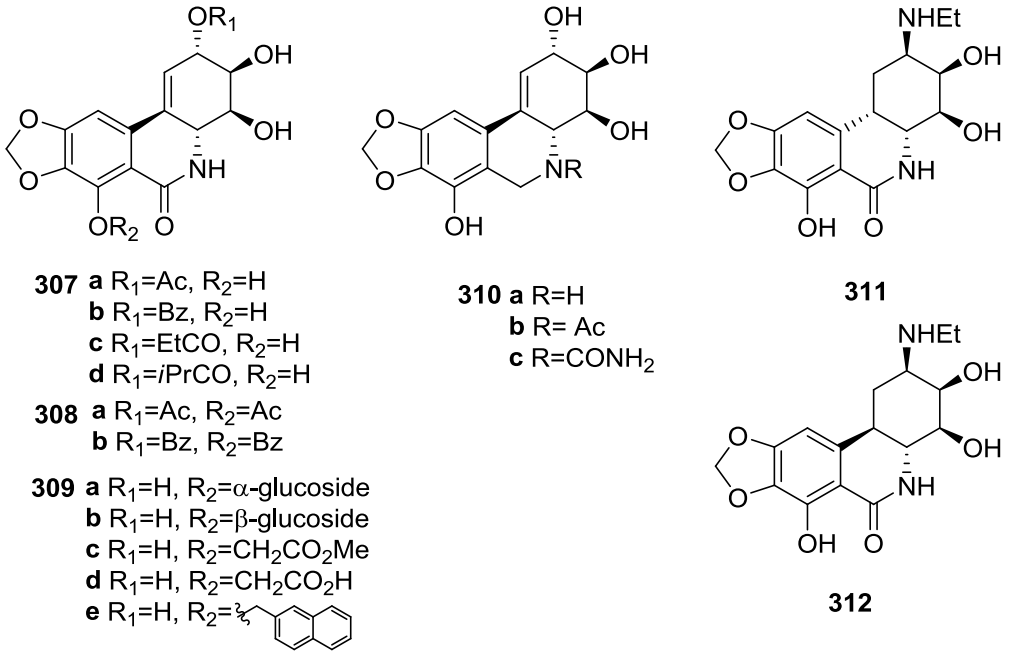


Figure 16. Narciclasine semisynthetic derivatives.

2.1.4. Biological studies and search for pharmacophore.

2.1.4.1. Biological effects and mechanism of action.

Members of Amaryllidaceae family have been recognized for a long time in folk medicine for the treatment of cancer related illnesses. Narciclasine was reported by Ceriotti[105] to have antimitotic activity on a murine sarcoma cell line (Sarcoma 180), as

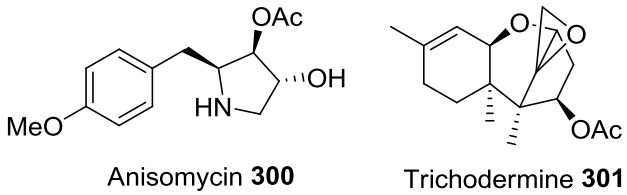
well as inhibition of growth of wheat grain radicles. Isolation of isocarbostyril family of Amaryllidaceae alkaloids was guided by anticancer assays, mainly by murine P-388 lymphocytic leukemia. These congeners have shown to have ED_{50} values as low as 0.01 μg/ml, they also exhibit activity against a wide panel of different cancer cell lines *in vitro*, as well as *in vivo*, particularly against the melanoma sub-family.[9]

The natural Amaryllidaceae isocarbostyril compounds **1**, **2**, and **10-13** displayed promising antiviral activity *in vitro* against flaviviruses such as Japanese encephalitis, Yellow fever and Dengue fever viruses, and bunyaviruses such as Punta Toro and Rift Valley.[96, 106] Futhermore, pancratistatin, narciclasine and 7-deoxypanratistatin have also shown some effect *in vivo* against mice infected with Japanese encephalitis, which constitutes one of the rare examples of non-immunomodulatory treatment of Japanese encephalitis.[96] Narciclasine was shown by McNulty[107] to be an inhibitor of CYP3A4 human cytochrome, while structurally similar *trans*-dihydronarciclasine does not exhibit inhibitory activity towards several types of human cytochromes. This study can be important for future preclinical development of drugs based on the isocarbostyril pharmacophore. Some antifungal activity of narciclasine[101] and antiparasitic activity of pancratistatin and 7-deoxynarciclasine[108] has been observed *in vitro*, unfortunately all activity in assays had a narrow therapeutic window in comparison with activity of these compounds against cancerous cells. Further discussion will be focused solely on anticancer properties.

Narciclasine and its congeners display several different mechanisms of action against cancerous and leukemia cells studies including apoptotis (programmed cell death), impaired cell proliferation, and decreased cell migration activity.[109, 110] Simultaneously,

these compounds show little or no activity towards healthy cell lines, therefore presenting the possibility that these compounds could be developed into selective anticancer agents and prospective drugs. Naturally, the mechanism of action of these molecules attracted significant attention from the chemical and biological communities, which can serve to deepen our knowledge about cell proliferation and differentiation between healthy and cancerous cells.

Early studies of the mechanism of action of narciclasine performed by Carrasco[111] demonstrated inhibition of eukaryotic ribosomal protein biosynthesis. These studies have been performed on *Saccharomyces cerevisiae* and rabbit reticulocytes (immature red blood cells). Experiments with ^{14}C and ^{3}H labeled peptides have shown, that narciclasine inhibits poly U-directed phenylalanine synthesis in ribosomes of rabbit reticulocytes as well as polypeptide synthesis in yeast polysomes, without affecting prokaryotic *E.Coli* cells. Furthermore, narciclasine may interact with peptidyl transferase in an eukaryotic large ribosomal subunit (60S) in the area known as the "anisomycin" area. It competitively binds to the same site as anisomycin (**300**) and to a lesser extent with trichodermin (**301**) – another potent protein synthesis inhibitor, Figure 17. Further evidence of a similar mode of action is the fact that a mutant yeast strain with resistance for **300** and **301,** also exhibits cross-resistance for narciclasine.[112, 113] Studies of the interaction of narciclasine and its semisynthetic derivatives were performed by Baez and Vasquez with tritium-labeled compounds[114] and further supported specific interaction of narciclasine with the 60-S ribosome subunit.



Anisomycin **300** Trichodermine **301**

Figure 17. Structures of inhibitors of cellular mechanisms.

One of the most interesting effects is the pronounced selective cytotoxicity of isocarbostyril congeners. It has been shown in the independent studies of Pandey[110] and Van Goietsenoven[109] that narciclasine and pancratistatin have a pronounced cytotoxicity and selectively induce apoptosis in cancer cell lines but are 200 times less toxic against normal cell lines. According to Pandey,[115] pancratistatin causes disruption of the mitochondria. He therefore reasoned that pancratistatin targets parts of the mitochondria, and their disruption activates apoptosis. Pandey postulated that the selectivity was caused by the ability of pancratistatin to differentiate between mitochondria of cancerous cells and normal ones.

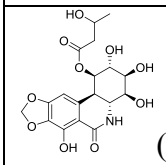
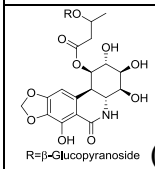
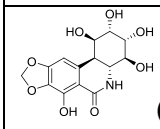
More recently, Van Goietsenoven proposed[109] that narciclasine (**1**) targets the ribosomal translation elongation factor eEF1A. This particular factor is responsible for the transport of aminoacyl tRNAs and the nuclear export of proteins. The disruption of eEF1A can cause irreversible damage and promote cell death. This hypothesis was supported by molecular docking studies, as well as binding studies on isolated human recombinant eEF1A and similar yeast elongation eEF2. Also, the fact that cancerous cells overexpress this particular factor can explain selectivity of narciclasine towards cancerous cells.

2.1.4.2. Anticancer activity of diverse analogues of Amaryllidaceae congeners

Initial studies towards refinement of cytotoxic pharmacophore of the Amaryllidaceae alkaloids relied on the comparison between compounds isolated from natural sources and those produced by semisynthesis. Seminal work on the refinement of pharmacophore was performed by Mondon and Krohn[7] which revealed that trans B/C ring juncture is essential for activity; *cis*-dihydronarciclasine (**276**) is less active by an order of magnitude then *trans*-dihydronarciclasine (**12**), whilst isonarciclasine (**277**) was inactive. The methylation of the hydroxyl at position C-7 reduces the activity of narciclasine. Unfortunately, no inhibition concentrations were reported in the article, however these values were presented later,[9] see Table 1, Table 3.

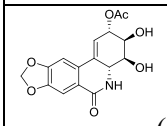
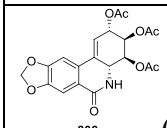
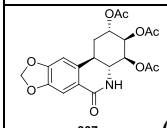
The evaluation of anticancer and antineoplastic activity of natural compounds, semi-synthetic and synthetic derivatives were performed by different groups on various cell lines, and the assays were reported with different units of measurement. It was decided to include only compounds, which constitute actual structural analogues, rather than prodrugs which undergo *in vivo* transformation to an active form. One of the most prominent examples are cyclic phosphates synthesized by Pettit,[100] which have shown relatively low inhibitory activity *in vitro* but were much more efficient on live models.[116] In order to make direct comparison of the results of assays, different values of inhibition from a variety of sources were compiled in a few tables, based on which part of pharmacophore was being altered. Data will be presented in the tables as mean values of all *in vitro* $IC_{50}/ED_{50}/GI_{50}$ values reported for the compound and normalised as μM.

Table 1. Activity of compounds isolated from natural sources.

Name of compound	Mean value of $IC_{50}/ED_{50}/GI_{50}$ (μM)	Difference from parent structure	*Ref.*
Narciclasine (**1**)	0.0155		[9]
Pancratistatin (**2**)	0.0955		[9]
trans-dihydronarciclasine(**12**)	0.0126		[9]
Lycoricidine (**10**)	0.145	Lacks 7-hydroxyl	[9]
7-deoxypancratistatin (**11**)	0.100	Lacks 7- hydroxyl	[26]
trans-dihydrolycoricidine (**13**)	0.0676	Lacks 7- hydroxyl	[9]
 (**313**)	0.91	3-hydroxybutric acid on C-1	[117]
 R=β-Glucopyranoside (**314**)	5.8	3(β-Glucopyranoside)-hydroxybutric acid on C-1	[117]
 (**315**)	4.3	Epimer on C-3 position	[118]

The removal of 7-hydroxyl consistently lowers the activity of all congeners by one order of magnitude. Hydroxyl on the position C-1 in pancratistatin is also deleterious to activity in about 5-10 times in comparison with narciclasine and *trans*-dihydronarciclasine. Relatively polar substituents on position **1** such as 3-hydroxybutyryl also lower activity, as well as the alteration of stereochemistry of C-3 hydroxyl.

Table 2. C-ring analogues.

Name of compound	Mean value of $IC_{50}/ED_{50}/GI_{50}$ (μM)	Difference from parent structure	*Ref.*
 **305** (**316**)	0.72	Acetate ester on position 2	[97]
 **306** (**317**)	1.01	Acetate ester on position 2, 3, 4	[97]
 **307** (**318**)	1.93	Acetate ester on position 2, 3, 4	[97]
307a	0.51	Acetate ester on position 2	[104]
307b	0.17	Benzoate ester on position 2	[104]
307c	0.04	Propanoate ester on position 2	[104]
307d	0.07	isobutiric ester on position 2	[104]
308a	0.75	Acetate ester on position 2, 7	[104]
308b	3.4	Benzoate ester on position 2, 7	[104]
309a	3.0	β-Glucoside on position 7	[104]
309b	Inactive	α-Glucoside on position 7	[104]
309c	16.7	Methyl acetate on position 7	[104]
309d	15.0	Acetic acid on position 7	[104]
309e	7.4	2-methylenenapthalene on position 7	[104]
310a	Inactive	Lacks amide	[104]
310b	Inactive	Lacks amide, acetate on nitrogen	[104]

310c	Inactive	Lacks amide, urea on nitrogen	[104]
311	Inactive	2-*epi* ethylamine	[104]
311	Inactive	2-*epi* ethylamine, cis-B/C junction	[104]
294	<0.0023	C-1 benzoate ester	[48]
193a	2.3	C-1 homologue alcohol, lacks 7-hydroxyl	[79]
192b	0.56	C-1 homologue acetate ester, lacks 7-hydroxyl	[79]
193a	Inactive	C-1 carboxylic acid methyl ester, lacks 7-hydroxyl	[79]
193b	Inactive	C-1 carboxylic acid, lacks 7-hydroxyl	[79]
297	0.018	*iso*butylamine on C-1	[103]
298	0.017	Benzylamine on C-1	[103]
299	0.03	Cyclohexylcarboxylic acid amide on C-1	[103]
300	0.007	benzoic acid amide on C-1	[103]
175a,b	Inactive	open C-ring, lacks C-3 hydroxyls	[76]
176	Inactive	Pyranoside replaced C-ring, lacks C-2, C-3, C-4 hydroxyls, C-1 *epi*	[76]
147	Inactive[a]	Lacks C-1,C-2, C-7 hydroxyls	[72]
197, 198	Inactive	**197** epi C-2, **198** epi C-2, C-4, both lack C-7 hydroxyl	[84]
133	Inactive	Lacks C-1,C-3, C-7 hydroxyls	[65]
126	2.3[b]	Lacks C-1,C-4, C-7 hydroxyls	[66]

[a] For all tested cell lines except, ED_{50} = 5.0 μM for murine leukemia P388.

[b] Average for 4 cell lines, results against NCI 60 panel was 2-3 magnitudes lower than **2**.

121	Inactive[c]	Lacks C-1,C-2, C-3, C-7 hydroxyls	[66]
***ent*-11**	10-fold less active than **11**		[72]
***ent*-1**, ***ent*-10**, **167**,	Inactive[d]		[119]
180	Inactive	Aromatic C-ring	[77]

Significant information on the pharmacophore was obtained during biological studies of C-ring analogues. Esterification of hydroxyls at positions C-2, -3, -4 decreases activity by one or two orders of magnitude, depending on substituents. Changes of stereochemistry at positions C-2, -3, and -4 of hydroxyl or absence of any of these groups significantly deteriorates activity, therefore confirming the importance of all three groups in the molecule in a specific stereochemical array. The installation of lipophilic groups at C-1 does not deteriorate activity, whilst polar groups, such as carboxylic group render compounds inactive. The notable example of the beneficial effect of lipophilic groups is C-1 benzoate (**294**). It is unclear whether ester **294** serves as a prodrug and just delivers pancratistatin to the active site, but the later discovery[103] of benzamide **300** and benzyl amine **298** with comparable activity shows that this aromatic ring might be a part of the pharmacophore.

Table 3. B-ring analogues.

Name of compound	Mean value of $IC_{50}/ED_{50}/GI_{50}$	Difference from parent structure	*Ref.*

[c] ED_{50}>153 μM for murine leukemia P388.

[d] 0-15% inhibition at 2μM for 7 cell lines.

	(μM)		
292	Inactive[e]	Lacks amide oxygen	[97]
292 hydrochloride	13.0	Lacks amide oxygen	[97]
293	5.7	Lacks amide oxygen	[97]
293 hydrochloride	7.7	Lacks amide oxygen	[97]
216	Inactive	Nitrogen replaced with oxygen	[88]
217	Inactive	Nitrogen replaced with oxygen, epi-stereochemistry of 4a	[88]
204	Inactive	No bond 10a-10b	[86]
236-238	Inactive	No amide fragment, open B-ring	[91]
210	Inactive	10b-epi configuration	[26]
222	12.0	Truncated B and C-ring	[26]
248a, 248b	Inactive	Truncated B and C-ring	[93]
iso-narciclasine (**277**)	11.8	Double bond 10b-4a	[9]
cis-dihydrolycoricidine	95.5	Cis B/C junction, lacks C-7-hydroxyl	[9]
cis-dihydronarciclasine(**276**)	3.8	Cis B/C junction,	[9]

In order to retain biological activity, the B-ring must remain unchanged. Any alteration in functional groups, such as replacement of amide moiety or truncation of rings led to complete disappearance of anticancer activity. Conversion of stereochemical relation of B/C ring from *trans* to *cis*-junction significantly reduces activity and unexpectedly

[e] IC_{50}>36 μM.

isomerisation of position of double bond to 10b-4a in isonarciclasine (**277**) renders compound inactive.

Table 4. C-ring analogues.

Name of compound	Mean value of $IC_{50}/ED_{50}/GI_{50}$ (μM)	Difference from parent structure	*Ref.*
254	12.5	Lacks C-7 hydroxyl, oxygen on position C-8	[72]
259	60.1	Indole replacement of A-ring	[82]
267	Inactive	TMS on position C-8,C-9	[95]
274	Inactive	Lacks C-7 hydroxyl, methylene dioxy bridge, hydroxyl on C-8	[85]
275	Inactive	Lacks C-7 hydroxyl, methylene dioxy, Carboxybenzyl on C-8	[85]

These results, however limited, nevertheless show that the significant changes in A-ring lead to decrease or complete disappearance of anticancer activity. Replacement of methylenedioxyarene fragment with bioisostere indole fragment[82] led to three orders of magnitude drop in activity. Removal of any of oxygenated substituents on position C-8/C-9 or replacement with substantially different functional groups also lowers activity. In fact, the only untested position for replacement or introduction of the new functional groups is C-10.

General information on the pharmacophore of the isocarbostyril group of Amaryllidaceae alkaloids with all these studies started to take shape. Two sites for possible modification,

without risk of significantly reducing activity, are positions C-1 and C-10. Therefore our goal is to design new efficient and general ways towards these two types on analogues.

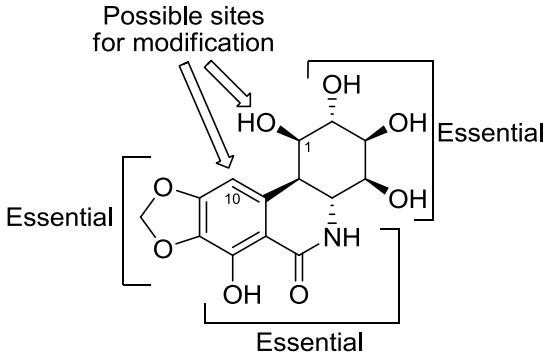


Figure 18. Model of pharmacophore presented on pancratistatin.

2.2. Aromatic dioxygenases

2.2.1. Discovery of aromatic dioxygenases.

The majority of animal and fungi organisms are capable of metabolizing aromatic compounds and transforming them to benign compounds *via trans*-diol **320** *via* transient intermediate epoxide **319**. It was believed that the bacterial degradation pathway involved the same intermediates, Figure 19. Gibson[120] was the first to show that some bacterial strains, namely *Pseudomonas Putida* F1, have an alternate aromatic degradation pathway. This pathway involves *cis*-dihydroxylation of the aromatic compounds for example toluene to the *cis*-cyclohexadiene diol (**316**), followed by oxidation by catecholde hydrogenase to catechol **317** and further degradation to non-aromatic compounds. This bacterial strain is able to utilize aromatic compounds as the sole source of carbon and energy.

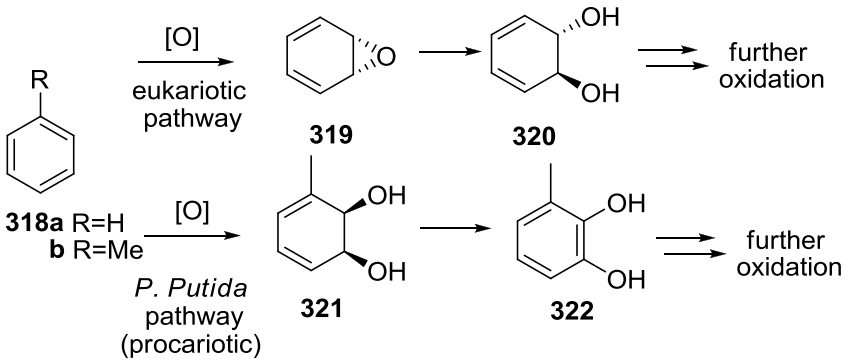


Figure 19. Possible oxidation pathways in eukaryotic and prokaryotic organisms.

Several years later, Gibson[121] created a mutant strain *Pseudomonas Putida* 39/D (*Pp* 39/D), which lacks the enzymes necessary for the oxidative transformation of *cis*-diol **321** to catechol **322** and therefore accumulates larger quantities of the transient metabolite **321**. This unusual type of 3,5-cyclohexadiene 1,2-diols (cyclohexadiene diols) is produced by bacteria with high enantioselectivity (> 98% *er*). The absolute configuration of **321** was proven by its oxidative degradation of to the known compounds.[122] In 1989 Gibson[123] identified the gene sequence responsible for the production of toluene dioxygenase enzyme (TDO) complex and transferred them into appropriate plasmid for functional expression in *E. Coli*. By designing this engineered strain, few major obstacles of biotransformation were avoided. First, the requirement for presence of certain aromatic molecules such as toluene or chlorobenzene as inducers of TDO expression was removed. The use of inducers with *Pp* 39/D inevitably led to contamination of the desired metabolite with cyclohexadiene diols, derived from inducers. Natural host of enzyme, *Pp* 39/D, has a relatively slow growth and expression rate of required enzyme, which decreases the space/time yield. The recombinant strain *E. Coli* JM109 (pDTG601), was created to overexpress the TDO enzyme upon treatment with sugar β-

isopropylthiogalactopyranoside (IPTG). Creation of this strain allows to streamline the production of cell mass and enzyme complex for biotransformation and significantly increases space/time yield of production of cyclohexadiene diols.

The structure of toluene dioxygenase (TDO) enzyme active site is unknown, even though X-ray crystal structure of the enzyme from the same family naphthalene dioxygenase (NDO) was solved.[124] Nevertheless, because of the large number of metabolites characterized, some empirical rules were established[125] and the stereochemical outcome of dihydroxylation of unsymmetrically substituted arenes can be predicted, Figure 20.

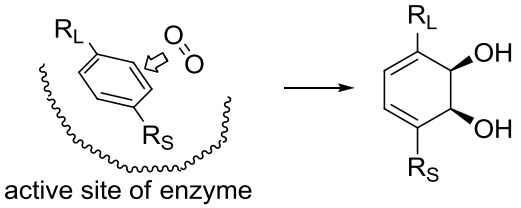


Figure 20. Boyd`s model for prediction of regioselectivity of dihydroxylation.

2.2.2. Utilization of diols in the natural product synthesis.

In a little over forty years since the first isolation and identification of stable *cis*-cyclohexadiene diols, more than 400 substrates produced by the dioxygenase family of enzymes were characterized. Only a small fraction of this library of richly functionalized compounds has been utilized in organic synthesis. All known metabolites and their applications for synthesis have been amply reviewed.[126-131]

Despite the acknowledgment by the biological community, chemists has been reluctant to include these new fascinating molecules into their manifold of chiral building blocks. The first example of application of benzene cyclohexadiene diol (**323**) in synthesis was that reported by Taylor[132] from ICI in 1983. He exploited radical polymerization of diene **323**,

followed by elimination of diol moiety towards synthesis of a polyphenylene polymer. The same year the synthesis of indigo from indole was reported by Gibson.[133] This synthesis also proceeded through the intermediacy of arene diol, but it was produced with the help of naphthalene dioxygenase-expressing organism. It is important to note that in both of these syntheses the diol functionality was destroyed in the process of forming product.

The first example of incorporation and exploitation of the diol functionality in synthesis was presented by Ley[134] almost nine years later after Gibson`s discovery in a short synthesis of pinitol (**325**). He used the steric hindrance of the protecting groups on the diol for facial selectivity in epoxidation reaction to produce epoxide **324**, Figure 21. Selective opening of the epoxide led to the protected tetraol **325**, which upon further functionalization provided racemic pinitol (**326**). The use of the arene diol functionality for steric guidance of further modifications as well as steric and electronic differentiation of alkene would prove to be standard strategies in the applications of these arene diols in organic synthesis.

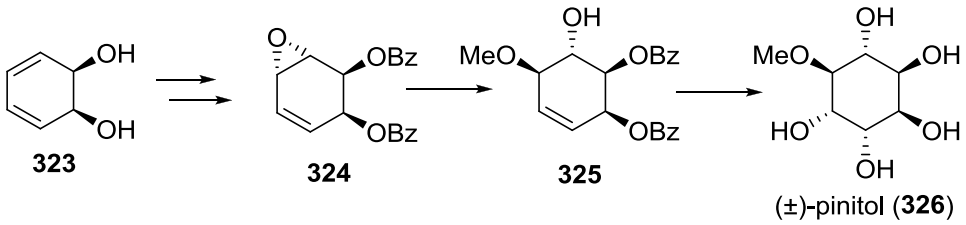
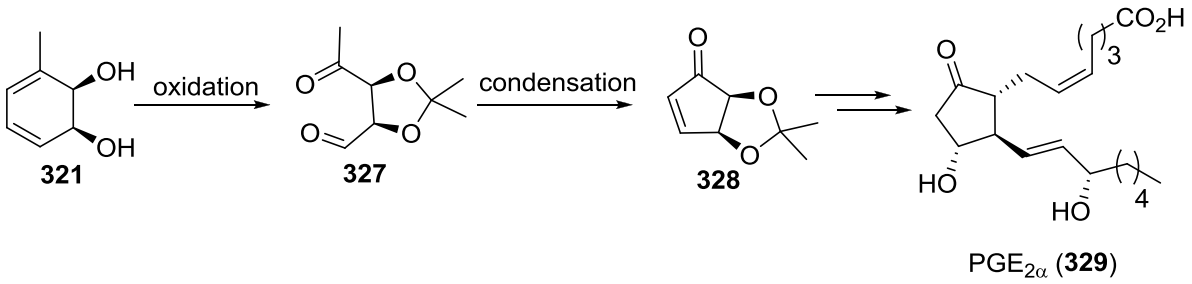


Figure 21. Ley`s synthesis of pinitol.

The next milestone in the application of the arene diols for organic synthesis occurred in 1988. The first utilization of asymmetric arene diol (**321**) in enantioselective synthesis was performed by Hudlický[135] in a formal total synthesis of prostaglandin E2

(PGE$_{2\alpha}$, **329**), Figure 22. The key step in the synthesis consists of the oxidative cleavage of both double bonds of protected diol **321** by means of ozonolysis. Ketoladehyde **327** was subjected to an intramolecular aldol reaction and provided enone **328**, a formal intermediate of prostaglandine E2 (**329**) synthesis, in only four steps.



321 **327** **328** PGE$_{2\alpha}$ (**329**)

Figure 22. Hudlický`s formal synthesis of prostaglandine E2.

Rich functionality of cyclohexadiene diols can be exploited in a many ways toward the synthesis of different classes of complex organic molecules. Key steps of some total syntheses will be presented with the emphasis on the diversity of approaches rather than diversity of targets.

One of the earliest and most explored transformations applied to the cyclohexadiene diols is the use of the diene moiety in the cycloaddition reactions. Gibson[121] was the first to report cyclohexadiene diols serve as diene components in the Diels-Alder reaction. Hudlický applied this approach extensively to the total synthesis of a variety of natural products such as zeylena,[136] conduramine A,[137] lycoricidine,[34, 35] and narciclasine.[26, 127] Last three syntheses share common steps, namely the tendency of diol **4** protected with the sterically demanding ketal group, to undergo facial and regioselective [4+2] cycloaddition with nitrosyl dienophiles.When applied to protected diol **330**, bicyclic oxazine **331** was formed, reductive cleavage of N-O bond with sodium amalgam of

which was followed by dehalogenation and retention of stereochemistry to provide protected conduramine **332**, Figure 23.

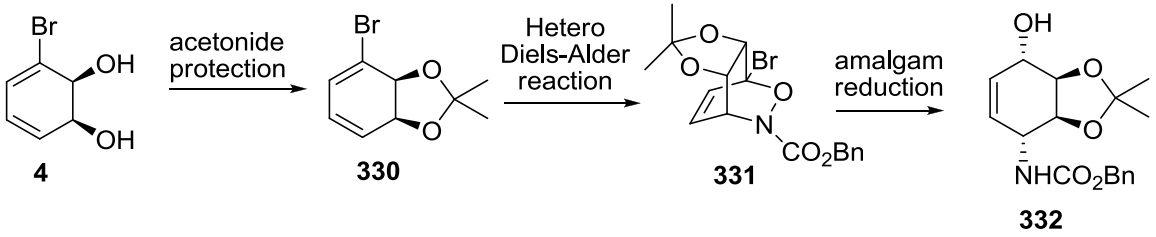


Figure 23. Hudlický`s approach towards conduramine-type compounds.

Alternative face selectivity of Diels-Alder cycloaddition reaction can be achieved upon performing reaction on unprotected cyclohexadiene diol. This strategy was utilized by Banwell[138] in the synthesis of (+)-armillavirin. Diol **321**, produced by dihydroxylation of toluene, was exposed to 19 kbar pressure in the presence of cyclopentenone (**333**) to provide tricyclic adduct **334**, Figure 24. Further lengthy modifications provided tricyclic ketone **335**, which upon subjection to photochemical conditions yielded the product of [1,3] acyl rearrangement, namely ketone **336**, as the major product. Few more transformations yielded natural sesquiterpenoid armillarivin (**337**).

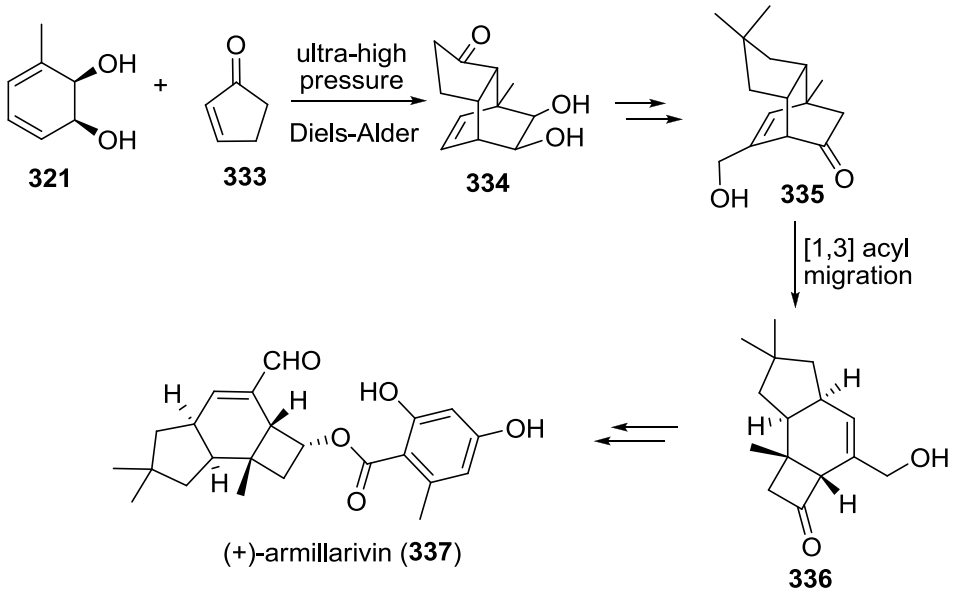


Figure 24. Banwell`s synthesis of armillarivin.

In addition to Diels-Alder type reactions, cyclohexadiene diols have also been employed in a variety of sigmatropic rearrangements and thermal transpositions, as well facilitated by metals. Two prominent examples of the latter transformations include reductive and oxidative allylic transpositions performed by Micalizio[139] and Hudlický.[140]

Micalizio utilized reductive titanium-mediated coupling of alkynes with allylic alcohol towards total synthesis of phorbasin C. The synthetic sequence started from cyclohexadiene diol **4** derived by oxidation of bromobenzene with TDO. Dihydroxylation of the less sterically hindered double bond was followed by radical dehalogenation to provide diol **339**, Figure 25. The key step involved the formation of metallacycle **338** from TMS-propyne followed by coordination with allylic hydroxyl in **339** and reductive transposition to provide 1,4-diene **340**, which was then transformed into final product (+)-phorbacin C (**341**).

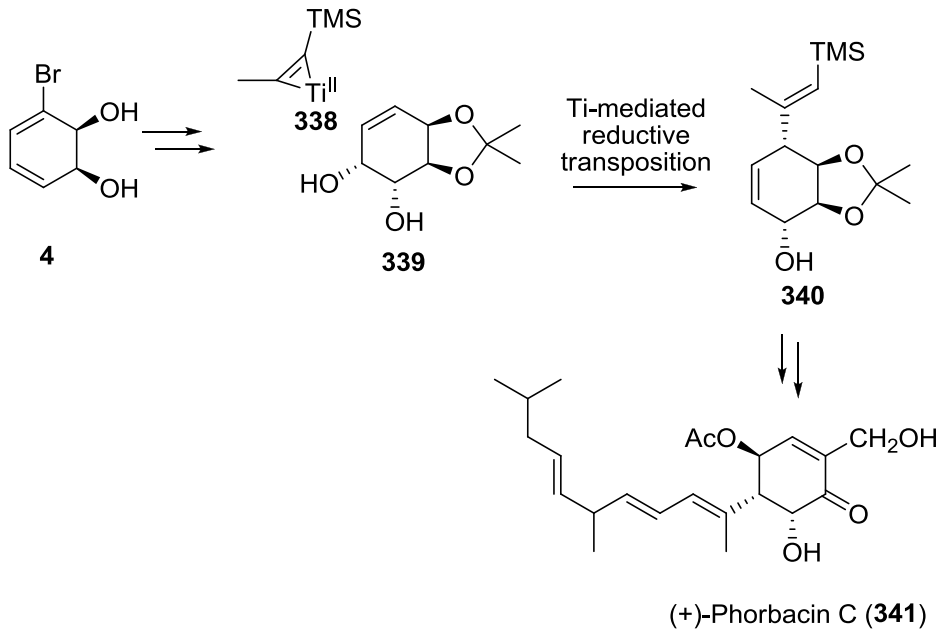


Figure 25. Micalizio`s synthesis of phorbacin C.

Oxidative allylic transposition was utilized by Hudlický in a short synthesis of the anti-viral drug Oseltamivir. The starting material for the synthesis was diol **342** produced from ethyl benzoate with moderate yield *ca.* 1g/L. Diol **342** was protected, submitted to cycloaddition to provide upon reductionof the N-O bond the key intermediate allylic alcohol **343**, which upon submission to oxidative transposition (Dauben-Michno reaction) mediated by chromium trioxide in acetic anhydride yielded enone **344**, Figure 26. Further transformations provided access to the Oseltamivir (**345**) in only seven one-pot operations.

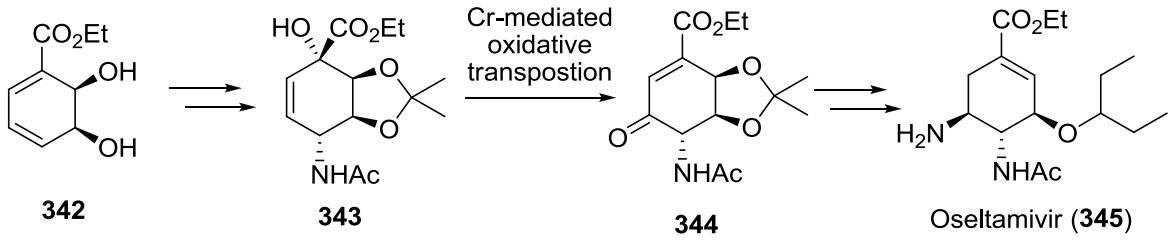


Figure 26. Hudlický`s synthesis of Oseltamivir.

Cross–coupling was also recognized and utilized as an efficient way to introduce new functionality to the halogen-containing cyclohexadiene diols. One of the most prominent examples is the utilization of iodo cyclohexadiene diol **346** by Banwell[141] towards a family of epoxyquinol-based natural products, Figure 27. In order to produce biosynthetic precursors of two complex natural products panepophenatrin (**349**) and hexacyclinol (**350**), iodo cyclohexadiene diol **346** was submitted to the sequential halobromination, Payne rearrangement, and Mitsunobu reaction to provide the key intermediate **347**. Stille coupling of enone **347** with different vinylstannanes provided two intermediates **348a** and **348b** which upon selective deprotection spontaneously underwent dimerization in the course intermolecular Diels-Alder reaction to form panepophenatrin (**345**) and hexacyclinol (**350**) respectively.

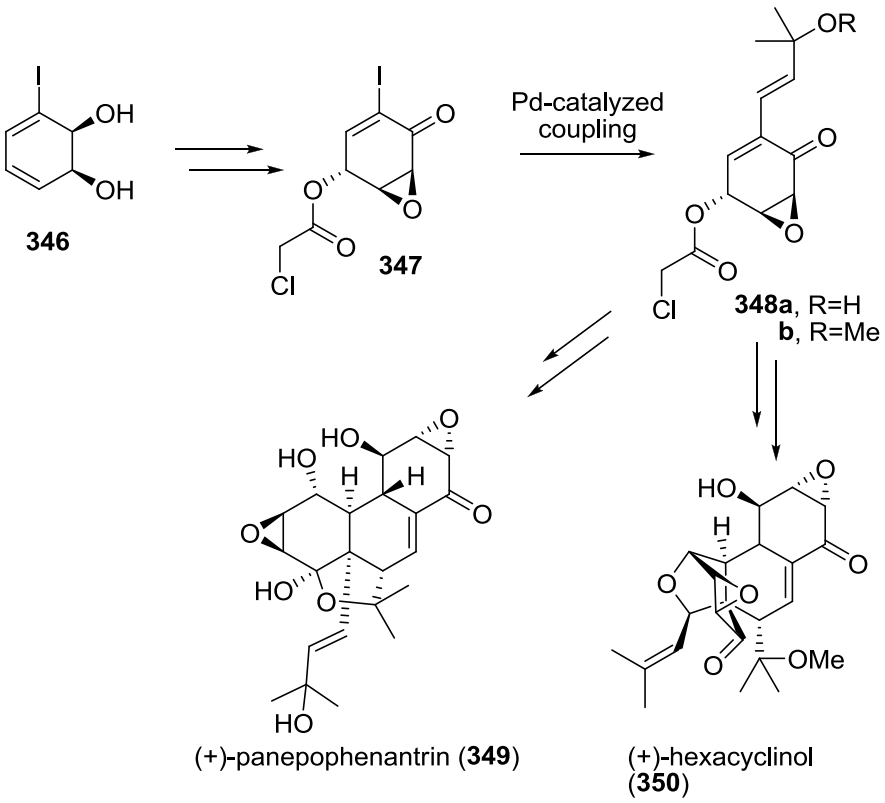


Figure 27. Banwell`s synthesis of panepophenantrin and hexacyclinol.

These examples of the synthetic utility of cylohexadiene diols only represent a fraction of known syntheses. Cyclohexadiene diols also have been effectively utilized in the synthesis of carbasugars,[142] morphinan[143-145] type alkaloids and many other classes of natural products.[126-131] Many targets attained from cyclohexadiene diols contain richly oxygenated amino cyclitol motif similar to those present in *Amaryllidaceae* alkaloids and therefore present an ideal starting point to develop a general and divergent strategy towards synthesis of different analogues of narciclasine and pancratistatin.

2.3. Polysubstituted pyridines

Pyridine ring systems are rarely observed in nature with only a limited number of complex natural structures containing a pyridine ring such as vitamin B_6 and nicotin.[146]

The pyridine scaffold, however is widespread in the pharmaceutical industry.[147-149] The biological activity of and wide range of applications for pyridine-containing compounds ensures synthetic interest and has led to the development of many approaches towards these fascinating molecules.

General approaches towards *de novo* synthesis of pyridines can be classified into three major categories: (i) cycloaddition, (ii) cyclocondensations, and (iii) rearrangements of different types of heterocycles, as shown in Figure 28. In this chapter the objective is not to give a comprehensive overview of all existing approaches, but the rather attention will be given to the application of more developed approaches to the synthesis of diversely substituted pyridines with the particular emphasis on polyoxygenated pyridines, because of their resemblance to the aromatic A-ring of the Amaryllidaceae alkaloids and hence the relevance to the topic of this dissertation. Some examples of syntheses of natural products containing polyoxygenated pyridine fragments will be presented in Section 2.3.4.

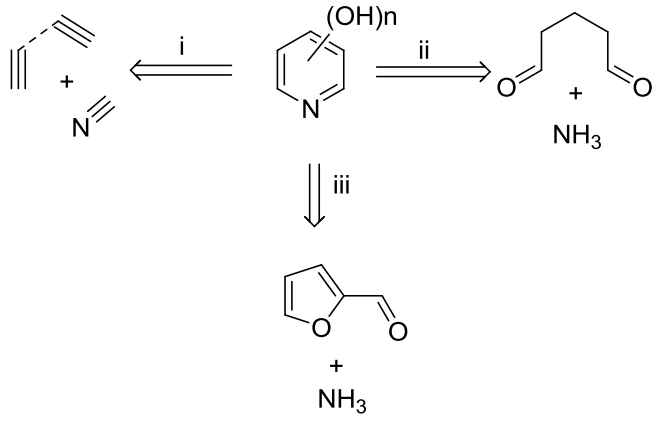


Figure 28. General routes towards pyridine ring scaffolds.

2.3.1. Cycloaddition reaction for the synthesis of polysubstituted pyridines

Cycloaddition methods offer many advantages in the synthesis of polysubstituted pyridine systems, since these transformations can be performed with high atom economy and their convergence allows, in principle, the regioselective preparation of pentasubstituted pyridines. One of the most widely used and prominent examples of a cycloaddition reaction is the [4+2] cycloaddition process also known as the Diels-Alder reaction. Nitrogen can be present in either the diene or the dienophile fragment of the ring to be formed and therefore three different retrosynthetic disconnections of the same hetero-Diels-Alder approach to pyridines are shown on the Figure 29. The first and one of the most developed approaches is the utilization of 2-azadiene with different dienophiles. Due to electron-withdrawing nature of nitrogen this reaction usually proceeds as an inverse electron demand Diels-Alder reaction. This reaction can be performed with isolated 2-azadiene but much more common is the application of different heterocycles bearing a 2-azadiene fragment and a leaving group such as 1,2,4-triazine (**352**) or oxazinone (**354**). Other versions of hetero-Diels-Alder reations include the reaction between 1-azadienes (eneimines) and alkene fragments, and diene fragment with azadienophile such as imine. Imine and 1-azadiene fragments can be easily produced *in situ* from nitrogen nucleophiles and aldehydes, thus rendering these Diels-Alder approaches similar to condensation reactions.

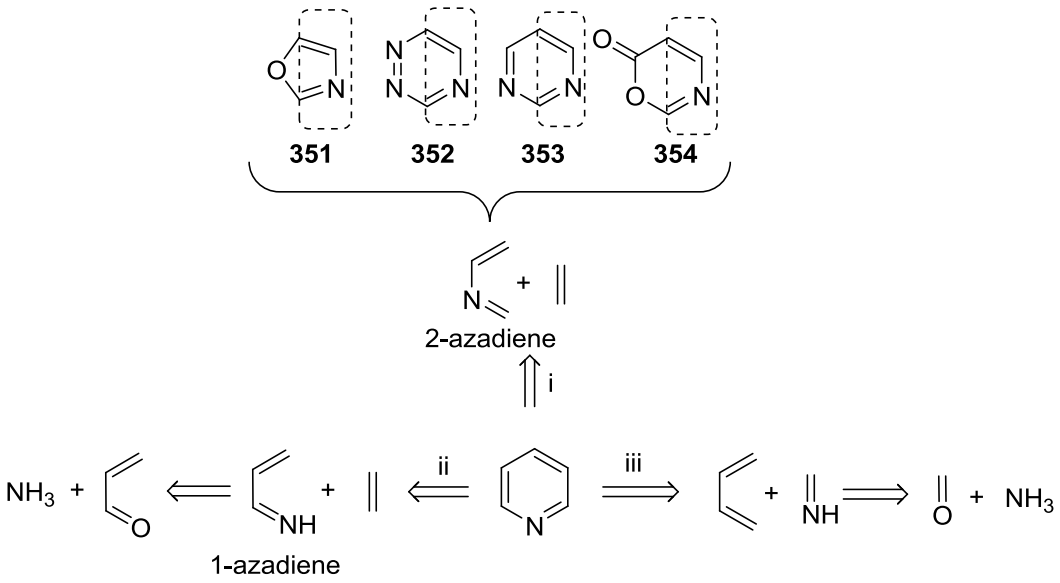
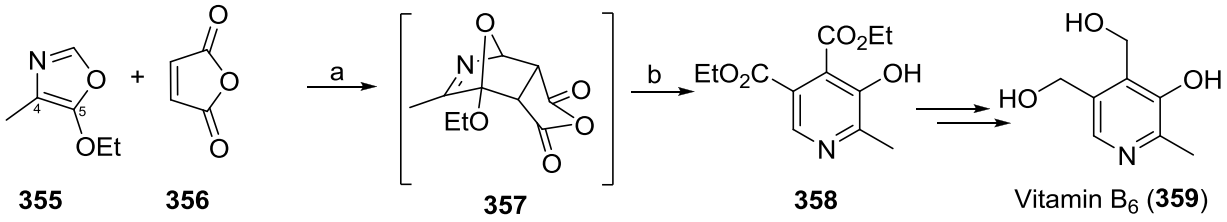


Figure 29. Three different variants of hetero-Diels-Alder approach to pyridine.

The hetero-Diels-Alder reaction was one of the earliest examples of a cycloaddition reaction, and its utility has long been recognized and extensively explored for the synthesis of natural products. One of the first examples of such a transformation was the use of oxazoles as azadiene fragments in what has become known as Kondrat`yeva reaction,[150] which was extensively used in the synthesis of vitamin B_6. Researchers from Merck developed their route[151] starting from 4-methyl-5-ethoxyoxazole (**355**) which was coupled with maleic anhydride (**356**), to produce tricyclic anhydride **357**, which upon treatment with anhydrous hydrogen chloride underwent ring opening and elimination yielded diester **358** in moderate yield, Scheme 42. Reduction of this pyridine yielded pyridoxine (**359**).



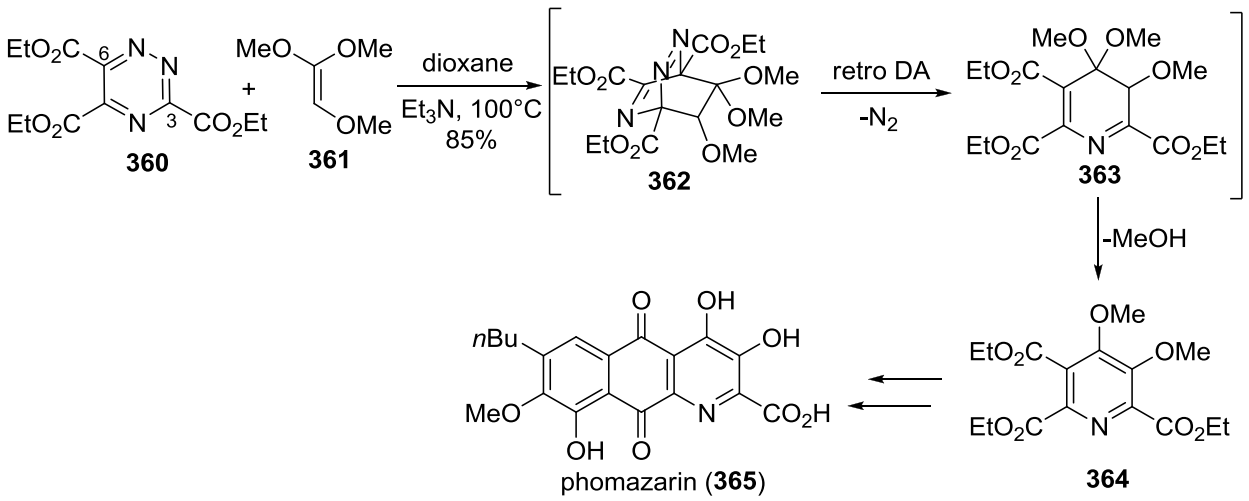
Reaction conditions: (a) benzene, 80°C; (b) HCl, EtOH, 25% for 2 steps.

Scheme 42. Example of pyridine ring construction from the synthesis of vitamin B_6.

A general way of production of such highly functionalised pyridines was used to generate libraries of polysubstituted pyridines by coupling a range of oxazoles with a variety of dienophiles.[152] However, one of the major limitations of this approach are the strict requirements on the substitution pattern of the oxazole ring. This reaction only proceeds successfully if the oxazole contains an electron donating group in the 5- position (usually an alkoxy group) and an alkyl substituent in position 4, therefore severely limiting the substitution pattern of the final pyridine.

Hetero-Diels-Alder reactions based on the use of 1,2,4-triazines as heterodiene fragments constitutes another well-studied approach towards polysubstituted pyridines. Early work in this field was performed by Neunhoeffer,[153] and extensively studied by Boger,[154] and, as a result, this particular transformation is sometimes known as Boger reaction.[150] The major difference in this particular transformation is that the presence of the three nitrogen atoms that make the diene component very electron deficient; as such, this process can be described as an inverse electron-demand Diels-Alder reaction and can be performed with electron-rich dienophiles such as enamines, enol ethers and strained alkenes. The cycloaddition usually occurs regioselectively between the C-3 and C-6 positions of the triazine. An example of such a transformation was utilized by Boger[155] towards the

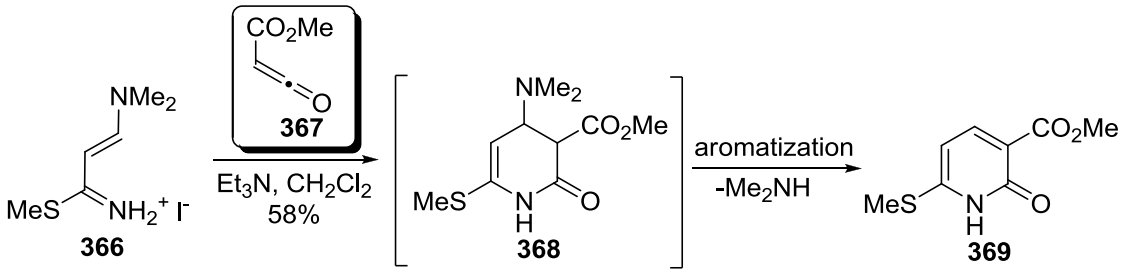
synthesis of phomazarin (**365**).The inverse electron-demand hetero-Diels-Alder reaction between the electron poor 1,2,4-triazine **360** and 1,1,2-trimethoxyethene (**361**) forms the transient bicycle **362**, which upon *retro* Diels-Alder elimination formed azadiene **363** and finally, upon elimination of methanol yielded pyridine ester **364** with good yield, Scheme 43. A major limitation of this approach, however, is the requirement of generating appropriately substituted triazines, which usually requires several steps.[156]



Scheme 43. Hetero Diels Alder approach towards the key intermediate in the synthesis of phomazarin.

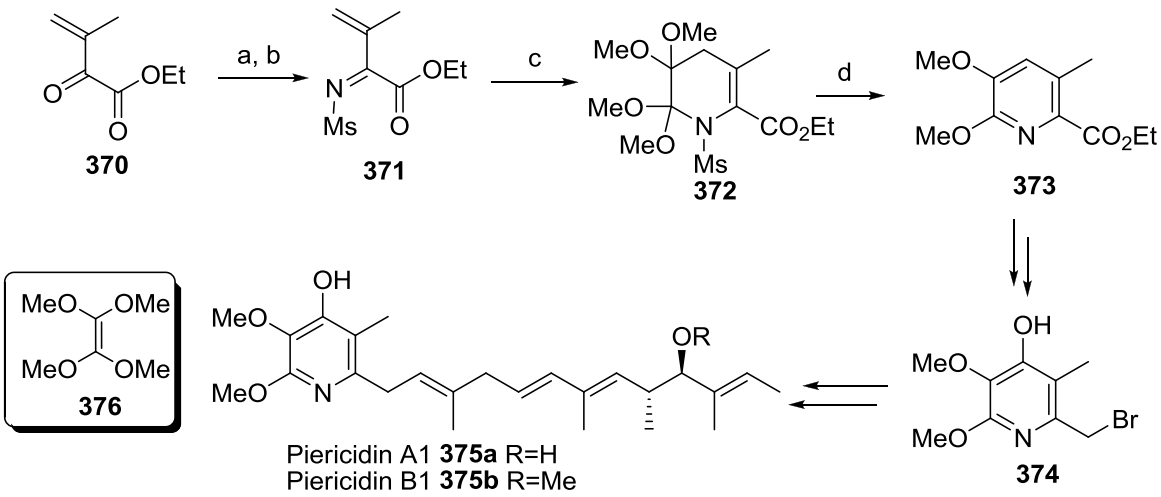
Alternative [4+2] approaches to a pyridine core have also been extensively studied and reviewed.[157] It must be noted that unsubstituted 1-azadienes (eneimines) are unstable and require stabilizing groups in order to prevent their tautomerization to the more stable enamines. Therefore, it is usually not eneimines, but eneoximes[158] and enehydrazones[159] that are used as diene fragments. Other eneimines equivalents have been described[160] such as dimethylaminoazadienium iodide **366**, which can undergo cycloadditions with

different types of dienophiles, including ketene **367**, followed by a elimination of the amine moiety and aromatization to produce substituted pyridone **369**, Scheme 44.



Scheme 44. 1-azadienes as cycloaddition partners in the synthesis of heterocycles.

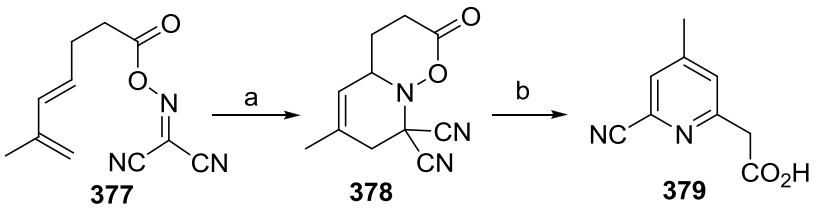
Potent inhibitors of electron-transport mitochondrial chain piericidins A1 (**375a**) and B1 (**375b**) and, subsequently, their analogues have been synthesized by Boger[161, 162]by applying the same strategy of an inverse-demand Diels-Alder cycloaddition of *N*-sulfonylimine **371**, Scheme 45. In order to perform this key transformation, the azadiene fragment was produced from α-carbonylester **370** by mesylation of the corresponding oxime. Reaction of azadiene **371** with tetramethoxyethene (**376**) led to tetrahydropyridine **372**, which upon treatment with Lewis acid eliminated methanol and yielded dimethoxypyridine **373**. Further transformation involved a directed ortho-metallation (DoM)/borylation/oxidation sequence to the install hydroxyl moiety, followed by reduction of the ester group and an Appel reaction to form pyridine **374**. Coupling this building block to the alkyl side chain and deprotection provided both natural products. The same building block was used towards the synthesis of analogues of these natural products.[157]



Reaction conditions: (a) NH_2OH*HCl, 96%; (b) CH_3SOCl, Et_3N; (c) **376**, PhMe, 64% for 2 steps; (d) BF_3*Et_2O, 88%.

Scheme 45. Boger`s synthesis of piericidins.

Pyridines can also be synthesized by a [4+2] cycloaddition between azadienophiles such as imines and nitriles and dienes.[163, 164] Additional electronic activation of imines is necessary in order to undergo a Diels-Alder reaction and this is usually achieved by treatment with Lewis acids such as $Yb(OTf)_3$, $SnCl_4$, $TiCl_4$, Et_2AlCl, *etc.*[164] Other possibilities, such as the use of oximes as imine substitutes, were successfully employed by Weinreb[165] for the synthesis of pyridylacetic acids. Malonoacyloxime **377** was shown to undergo thermal cycloaddition under high dilution to form bicycle **378**, Scheme 46. Base promoted elimination of cyanide from oxime **378** led to aromatization and formation of pyridylacetic acid **379** good yield.



377 378 379

Reaction condtions: (a) PhMe, reflux, 62%; (b) Cs_2CO_3, DMF, 67%.

Scheme 46. Weinreb`s synthesis of pyridylacetic acids.

Another attractive and atom-economical approach towards polysubstituted pyridines is their production *via* [2+2+2] cycloaddition of alkynes and nitriles. In principle, this process is symmetry allowed but negative entropy barriers of approximation of three different fragments and the activation energy makes purely thermal cycloaddition of alkynes and nitriles a rare case. In fact, there is only one example of a metal-free transformation of this type, and it is not an actual [2+2+2] cycloaddition, but rather a case of an intramolecular sequential ene/Diels-Alder reaction.[166] The vast majority of these cycloadditions are performed with a metal catalyst, which assists the pre-coordination of unsaturated fragment, and lowers entropic and enthalpic barriers. The general mechanism of such heterocyclic [2+2+2] cycloaddition is similar to their benzene counterparts. A significant number of reviews on the topic of pyridine formation *via* [2+2+2] cycloaddition have been published.[167-169]

The first reported case of a cycloaddition for the synthesis of pyridine was described in 1876 by William Ramsay[170] by passing the mixture of acetylene and hydrogen cyanide through a red-hot iron tube. However modern metal-catalyzed methods allow for this transformation to occur under significantly milder conditions. Rediscovery of this cycloaddition was observed with complexes of cyclopentadienylcobalt by Wakatsaki and Yamazaki,[171] and therefore these complexes are explored often for promoting such

cycloadditions and cobalt is the catalyst of choice because of its low cost, wide scope and substrate tolerance.

The general mechanism of the cobalt-catalyzed cycloaddition was studied by Bönneman[172, 173] and is displayed on Figure 30. Mechanistic details are similar to those in the formation of a benzene ring. The first step generates the catalytically active species **380** from stable precatalyst $CpCoL_2$ which coordinates with two alkyne fragments. The coordinated alkynes undergo reversible oxidative coupling with formation of metallocyclopentadiene **381**. In the last step the unsaturated metallocycle reacts with the nitrile or other alkynes (unproductive pathway). After an insertion (**383**) or cycloaddition (**384**) reaction with nitrile, these intermediates undergo a reductive elimination of the reactive metal species and yield the pyridine product **385**. There are a few other mechanisms that have been proposed, but this particular one is supported by the isolation of reactive intermediates and their independent reaction with alkynes.

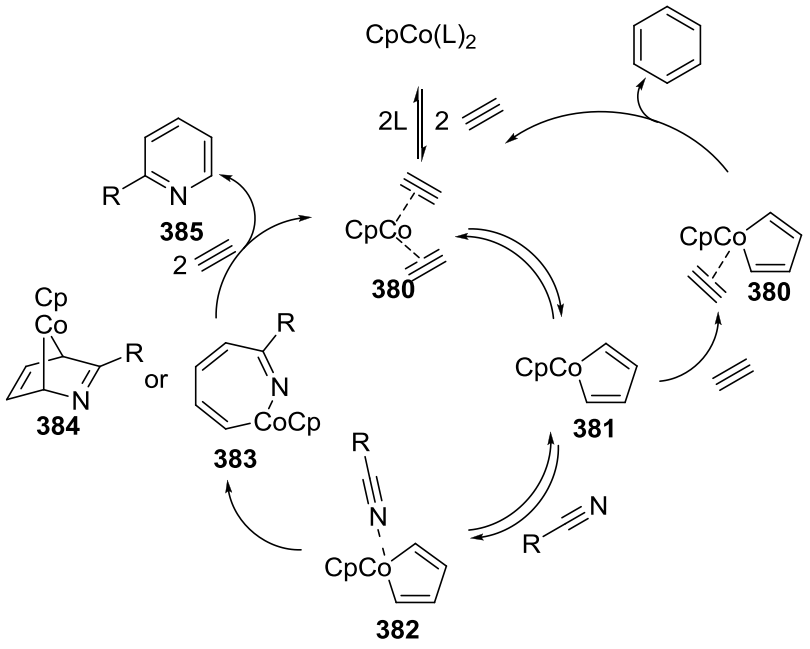


Figure 30. Catalytic cycle of Co-catalyzed [2+2+2] cycloaddition.

Although many examples of the cobalt-catalyzed reaction have been reported, the intermolecular regioselective assembly of two unsymmetrical alkynes with a nitrile to yield a single pyridine isomer remains an unsolved synthetic challenge. Major competing side-reactions are the oligomerization and trimerization of the alkynes. This obstacle can be overcome by performing the reaction in the presence of an excess of the nitrile component and using non-terminal alkynes with bulky protective groups, such as trimethylsilyl. A significant problem is the competitive formation of two pyridine regioisomers. Upon the addition of the monosubstituted alkyne to the nitrile it is common to see formation of both 2,4,6-(**389**) and 2,3,6-substituted pyridines (**390**), Figure 31. An investigation of different catalysts has been conducted,[174, 175] and it appears that electron-rich ligands provide moderate regioselectivity and poor yields, while electron-poor ligands provide good yields low regioselectivity.

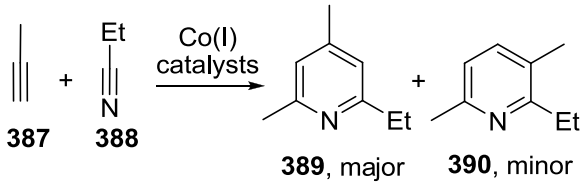
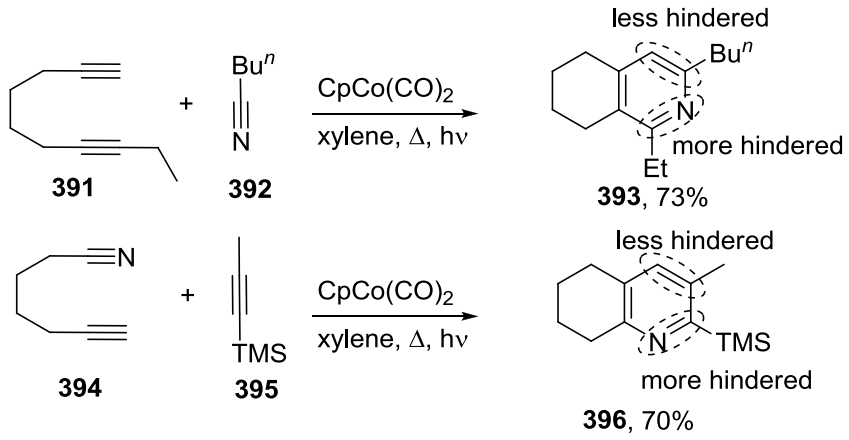


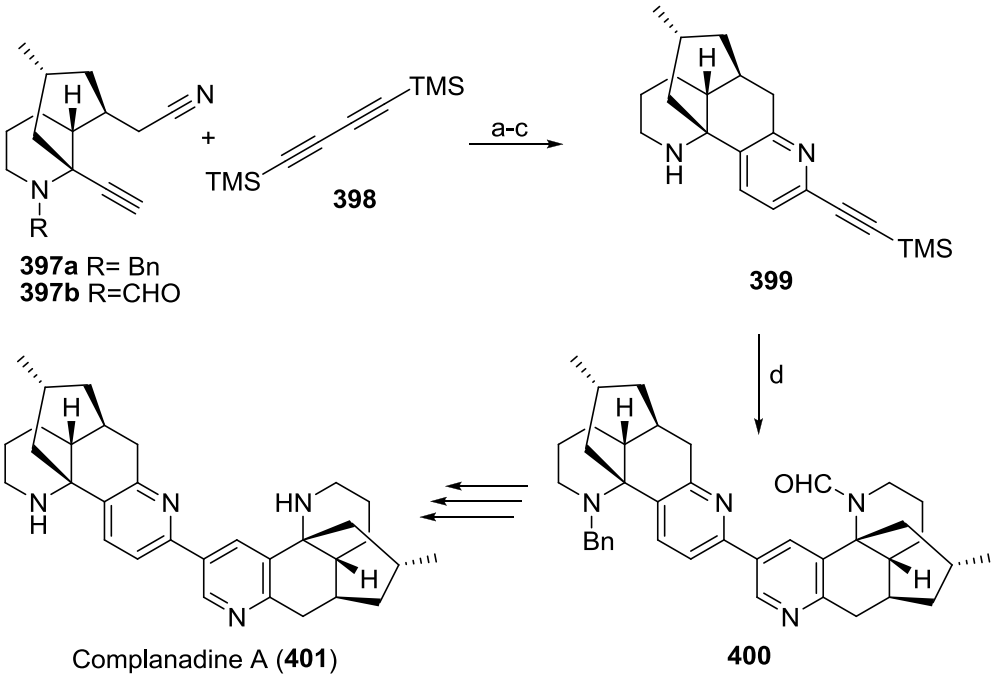
Figure 31. Regioselectivity in the reaction between propyne and propionitrile.

The best way to circumvent this regioselectivity issue is to perform the coupling on tethered α,ω-diynes (**391**)[176] or ω-cyanoalkynes (**394**),[177] with a third building block (nitrile or alkyne respectively). Therefore the bicyclic products such as **393** and **396** can be produced in a regioselective manner, Scheme 47. The reaction proceeded well with electron-rich alkyl and aryl nitriles, with 17:1 regioselectivty for **387**, but unfortunately, the reaction scope is somewhat limited, and electron poor nitriles do not undergo cycloaddition in good yields. In the case of cyanoalkyne **394** only one isomer was formed. The regioselectivity of the pyridine ring formation can be predicted, based on the formation of metallocyclopentadiene intermediate between two alkynes, followed by such addition of nitrile, the nitrogen of the pyridine ring will form a bond with the most hindered substituent of alkynes.



Scheme 47. Regioselectivity of the cycloaddition on tethered systems.

One of the most recent and elegant application of the cobalt-catalyzed cycloaddition was utilised by Siegel[178] for the synthesis of complanadine A (**401**). The challenge of this pseudo-dimeric structure was addressed by utilizing a strategy of sequential regioselective additions of TMS-diyne **398** to α,ω-cyanoalkyne **397a**, Scheme 48. The first [2+2+2] cycloaddition proceeded under the standard conditions in the presence of one equivalent of cyclopentadienyl cobalt dicarbonyl ($CpCo(CO)_2$) in high yield and with excellent regioselectivity (25:1) to provide pyridyl alkyne **399**. After completing the first cycloaddition, desilylation of the pyridine ring was performed and the second cycloaddition was observed to have a different regioselectivity, due to the addition of PPh_3 and utilization of a different building block **397b**. After further deprotection steps, the synthesis of the final bipyridyl natural product **401** was completed.



Reaction and conditions: (a) $CpCo(CO)_2$ 100 mol%, THF, 140°C, 82%; (b) TBAF, THF, 85%; (c) LHMDS, TMSCl, 90%; (d) **397b,** $CpCo(CO)_2$ 300 mol%, PPh_3, 1,4-dioxane, 140°C, 56%.

Scheme 48. Siegel`s synthesis of complanadine A.

Other building instead of nitriles blocks that can be successfully utilized for the synthesis of pyridines are heteroallenes such as isocyanates. Earl and Vollhardt[179] applied this particular transformation to the total synthesis of the cytotoxic quinoline alkaloid camptothecin **405**. In order to complete the formation of the pyridone ring, protected α,ω-isocyanatoalkyne **402** was submitted to a cycloaddition with the silylated pentyne **403** using $CpCo(CO)_2$, Figure 32. Regioselective formation of a single pyridone **404** was observed, which upon further modification provided for two formal approaches towards racemic camptothecin (**405**).

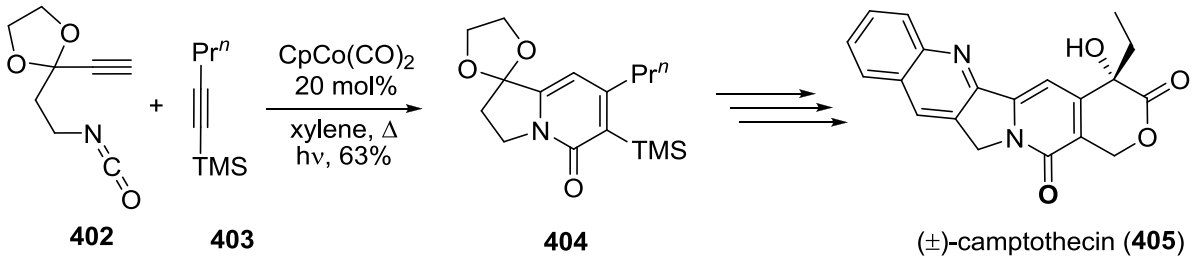


Figure 32. Vollhardt`s synthesis of camptothecin.

The original conditions of the cobalt-catalyzed cycloaddition were somewhat harsh, and consisted of refluxing catalyst $CpCo(CO)_2$ and the substrate in xylenes.[171] Prolonged exposure to heat and the thermal instability of this catalyst was somewhat overcome by making use of excess of the catalyst (up to 100 mol. %) and/or syringe pump addition of a mixture of the substrate and the catalyst to the boiling solvent. For example, Vollhardt`s synthesis[180] of lysergic acid derivatives utilised the cycloaddition of silypropyonamide **407** with indolonitrile **406** as a key strategy, Figure 33. In order for this reaction to proceed successfully 1 equivalent of catalyst was required, prolonged exposure to heat and irradiation led to desilylation and low yield of cycloaddition product, pyridine **408**.

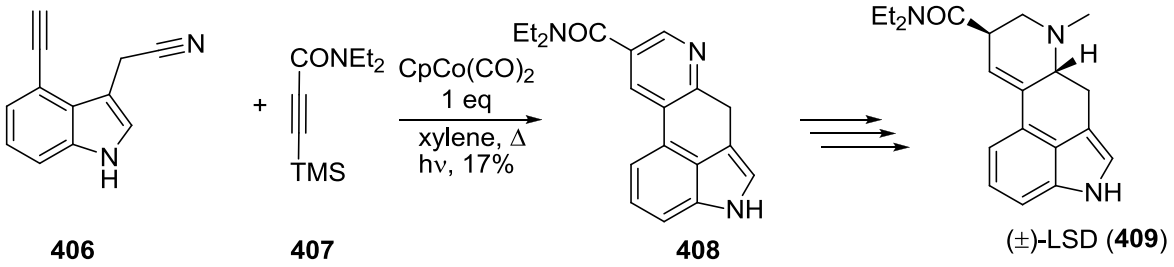


Figure 33. Vollhardt`s cycloaddition approach towards lysergic acid derivatives.

As can be seen, a prolonged exposure to the boiling solvent led to a significant decomposition of thermally unstable products, especially silylated pyridines, and

therefore milder ways of activation of the catalyst were sought. Activation of more weakly-bound complexes such as **414** and **415** can be achieved in milder conditions, thus the cycloadditions may be performed in more environmentally benign solvents such as water.[181] This approach has been used by Eaton[182] to create library of polysubstituted pyridines such as **412**, by means of a cycloaddition between nitriles and 1,4-butynediol (**410**) in water-methanol mixture in the presence of water-soluble catalyst **413**, Figure 34.

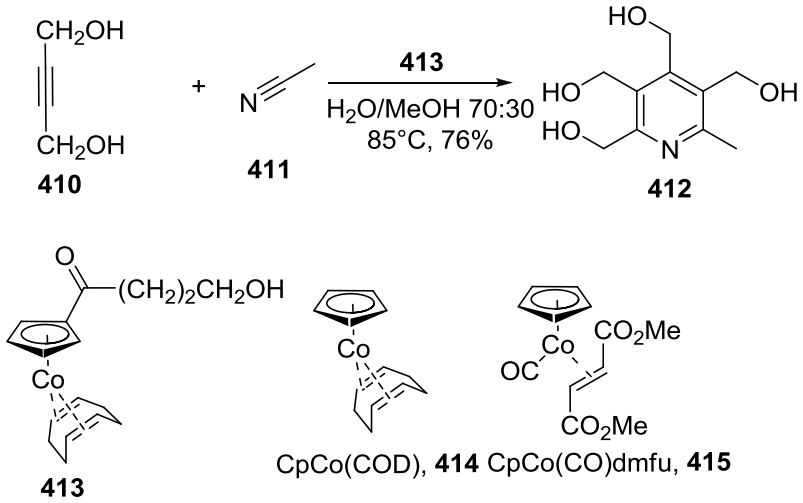


Figure 34. Cycloaddition reactions in water using new cobalt catalysts.

CpCo(COD) (**414**) also was shown to be a competent catalyst at room temperature upon irradiation with UV-visible light (350-500 nm).[169] Sensitive chiral nitriles[183] underwent cycloaddition under these mild conditions without significant racemization. Another approach to improve the yield and reduce the decomposition of sensitive substrates is to shorten the reaction time, which can be achieved by applying microwave conditions.[184] Recently, a more stable and efficient catalyst was developed by Malacria and Gandon.[185, 186] Most of the cobalt (0) catalytic systems are air-sensitive and therefore require special handling techniques as well as degassed solvents; however, it was discovered that the

complex of cobalt cyclopentadienyl with electron deficient alkenes, such as dimethyl fumarate, namely CpCo(CO)dmfu (**415**), can efficiently catalyze the transformation under mild thermal conditions and even be recycled after the reaction.[178]

Other transition metals can also catalyze [2+2+2] cycloadditions and might represent viable alternatives to the widely used cobalt method. These metals, especially ruthenium and rhodium, despite their cost, offer a distinctively different electronic preference for substituents and therefore offer complementary approach to design of reaction. For example, ruthenium (II) catalysts such as Cp*Ru(COD)Cl,[187-189] and $[Cp^*Ru(MeCN)_3]PF_6$[190] can catalyze the reaction between 1,6-diynes and electron poor nitriles such as cyanoesters, and α-halonitriles, something which cannot be attained cobalt catalysis. Low valent nickel[191] and iron[192] catalysts showed opposite regioselectivity of cycloaddition to that expected from the common cobalt-catalyzed cycloadditions described above. Recent discoveries in the field of cationic rhodium complexes[193] have shown that the [2+2+2] cycloaddition can be performed at room temperature in the presence of rhodium(I) and BINAP-type ligands. For instance, the regioselective cycloaddition of terminal electron-rich alkynes such as **416** with electron deficient nitriles as well as electron-rich nitriles was discovered by Tanaka,[194] Figure 35.

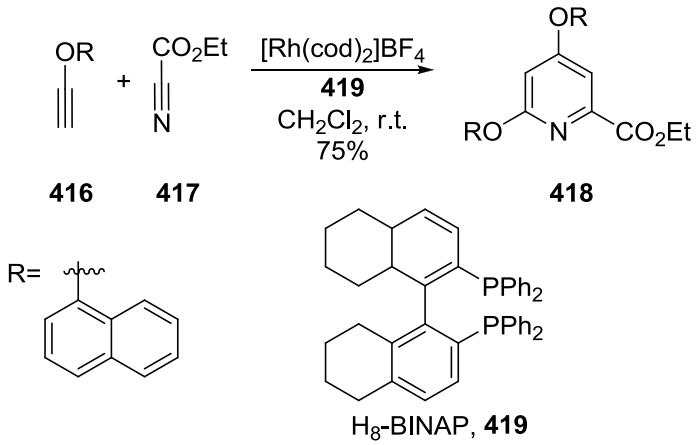
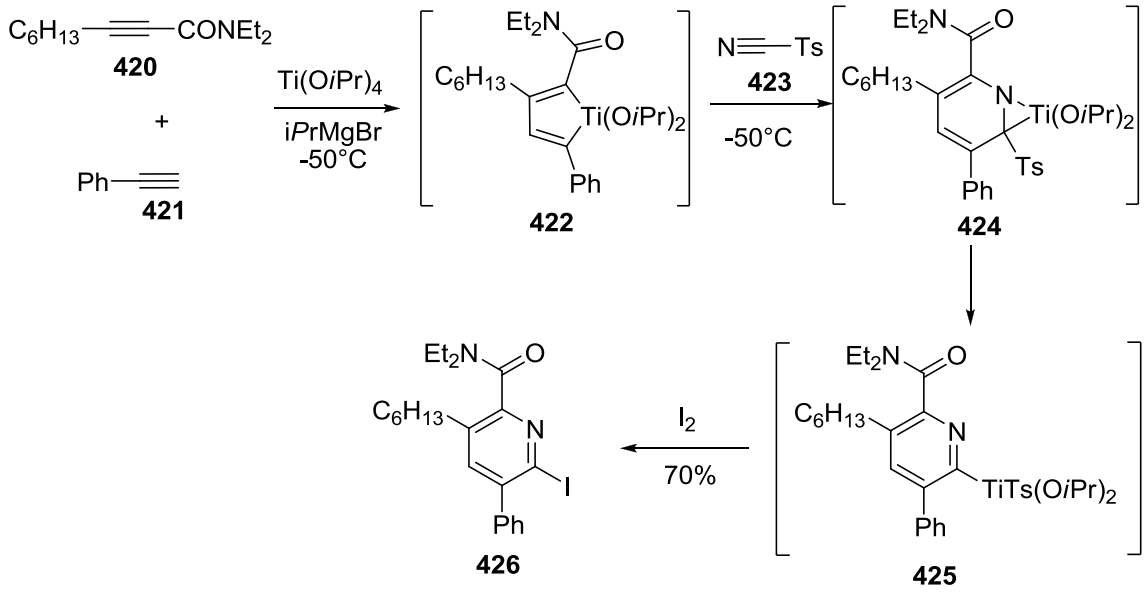


Figure 35. Rhodium(I)-catalyzed cycloaddition for the formation of polyhydroxypiridines.

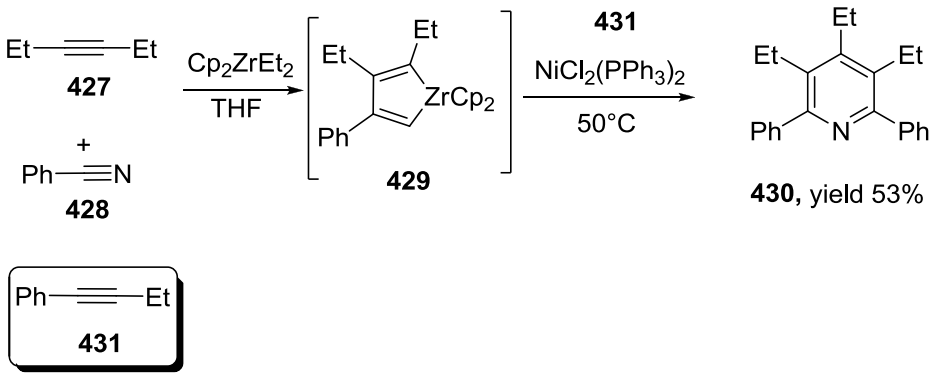
One distinctive case of the metal-facilitated [2+2+2] cycloaddition is the use of complexes of low-valent titanium and zirconium for the construction of the pyridine ring. The main difference lies in fact that it is not an actual catalytic process but rather the nucleophilic addition of organometallic reagents to unsaturated carbon-carbon and carbon-nitrogen bonds. Because of different mechanism, this particular type of cycloaddition can result in a stepwise regioselective coupling of three unsymmetrical fragments, something which cannot be achieved selectively with any other transition metal.

Sato[195] utilized divalent titanium species generated *in situ* and similar to intermediates in the Kulinkovich reaction. Due to high oxophilicity of titanium, it coordinates regioselectively to the alkylpropiolic amide **420** and phenylacetylene **421** to form adduct **422**, Scheme 49. Addition of tosylcyanide (**423**) to the metallo-cyclopentadiene intermediate **422** proceeds smoothly and after series of rearrangements the titanium intermediate **425** can be quenched with a variety of electrophiles, including proton, halogens, or alkyl halides, providing tetrasubstituted pyridines in one step in good yields.



Scheme 49. Sato`s synthesis of polybubstitued pyridine.

A similar case was observed for zirconium facilitated reaction. Takahashi[196] studied the formation of azametallocyclopentadienes **429** upon treatment of internal alkynes and nitriles with low-valent zirconium species. The formed intermediates undergo coupling with different alkynes in the presence of nickel dichlorodiphosphine to produce pentasubstituted pyridines, Scheme 50. Unfortunately, this reaction was observed only with alkyl- and aryl-substituted alkynes and nitriles.



Scheme 50. Takahashi`s synthesis of pentasubstituted pyridines.

All of these improvements extended the scope of the reaction and allowed for the formation of the pyridine core under much milder conditions and with improved regioselectivity, but nevertheless, cobalt remains the first catalytic metal of choice for the [2+2+2] cycloaddition in the synthesis of complex natural products.

2.3.2. Condensation reaction for the synthesis of polysubstituted pyridines

Historically, a condensation route is one of the earliest to be discovered and the most developed route towards polysubstituted pyridines. Generally it consists of the reaction between carbonyl compounds of different degrees of unsaturation and a nitrogen source such as a primary amine or ammonia. An overview of different retrosynthetic routes is presented in Figure 36. It is not to any extent a comprehensive representation of all existing condensation methods, which can be found elsewhere,[150, 197] but rather examples of syntheses which lead to differently substituted hydroxypyridines.

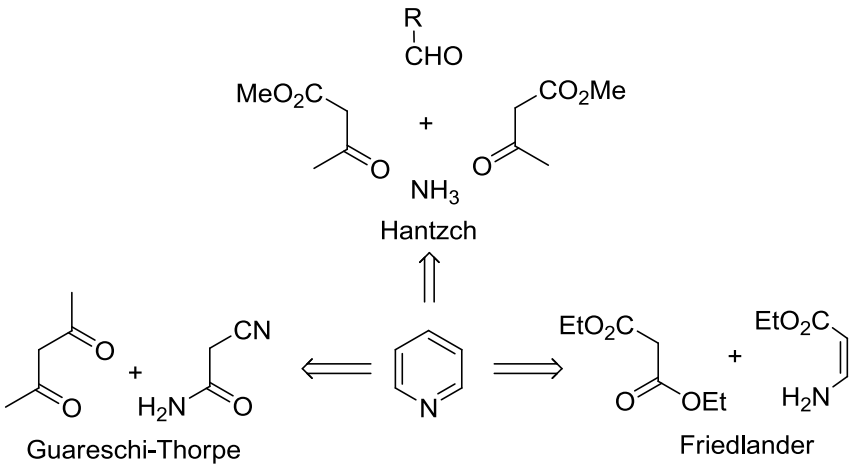


Figure 36. General condensation strategies towards pyridine core.

The Hantzsch reaction is one of the oldest multicomponent reactions and it consists of a condensation between two 1,3-dicarbonyl fragments **433**, ammonia and aldehydes **435**, Figure 37. This reaction provide access to a wide range of 1,4-dihydropyridine compounds **435**, which can be oxidised to the corresponding pyridines **436**. The scope of the classical Hantzsch reaction is somewhat limited by the fact that the substitution pattern of the product will always bear carbonyl/ester substituents at the positions C-3 and C-5.

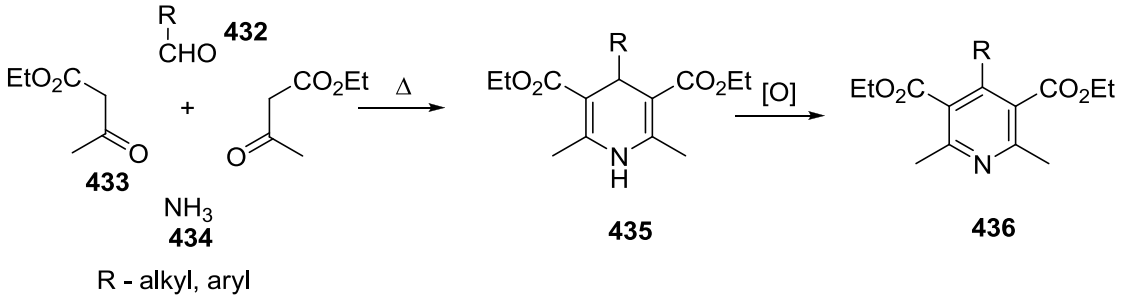


Figure 37. Hantzsch synthesis of pyridines.

New modifications of this transformation have been developed[198] to overcome the problem in the synthesis of unsymmetrical pyridines. One way to approach it is to utilize preformed enamines such as β-aminocrotonate **439** and its reaction with ketoester **438** and aldehyde **437** in order to produce dihydropyridine **440** with high yield, Figure 38.

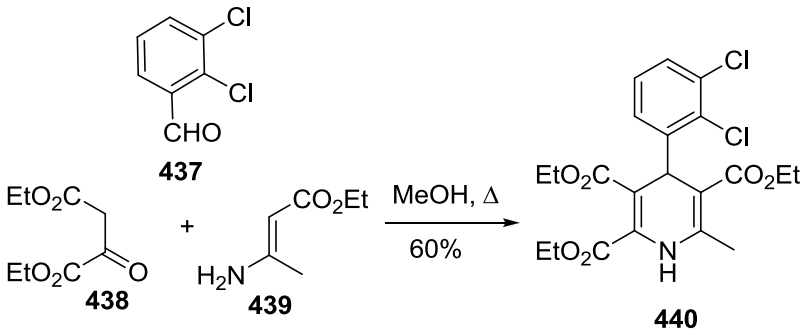


Figure 38. Modified Hantzsch synthesis.

The related Guareschi-Thorpe synthesis is a convenient way to generate polysubstituted 2-pyridones. It is conceptually similar to the Hantzsch pyridine synthesis with the difference that cyanoacetic derivatives are used as one of the building blocks instead of one of the 1,3-dicarbonyls. For example, in order to assemble 2,6-dihydroxypyridine **443**, cyanoacetamide (**442**) was subjected to reflux in the presence of methylacetoacetate(**441**) and the basic amine in methanol to provide 2,6-dihydroxypyridine **443** in good yield, Figure 39.

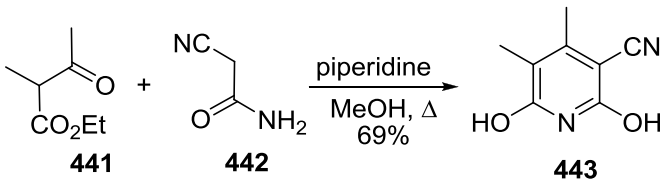
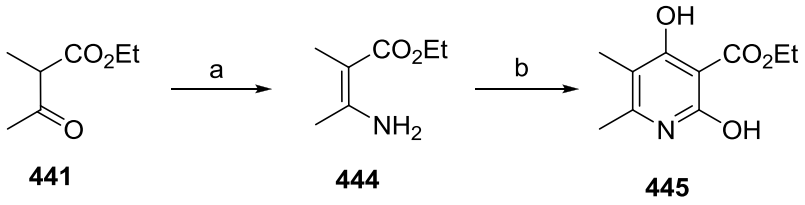


Figure 39. Guareschi-Thorpe condensation.

The Friedlander condensation is one of the most common and reliable methods for the preparation of 2-hydroxypyridines. It consists of the condensation between β-aminocrotonate **444** and 1,3-dicarbonyl compounds, Scheme 51. Deshong[199] applied this method towards the synthesis of 2,4-dihydroxypyridine **445**. The synthesis started from methylacetoacetate **441**, which was converted to aminocrotonate **444** upon treatment with

ammonia. The formed enamine **444** was submitted to the reaction with diethylmalonate in basic conditions led to desired dihydroxypyridine ester **445**.



441 **444** **445**

Reaction condtions: (a) NH_3, Bentonite K-10, 93%; (b) $CH_2(CO_2Et)_2$, NaOEt, EtOH, PhMe, 69%.

Scheme 51. Friedlander condensation.

2.3.3. Isomerisation of different heterocycles and acyclic compounds

Oxygen-containing five and six-membered heterocycles such as furans, pyrilium salts and 2-/4-pyrones have been successfully used for the synthesis of pyridines and especially hydroxypyridines/pyridones. Aminolysis of readily available 2-furan carbonyl derivatives of type **446** leads to nucleophilic ring-opening/ring-closure sequence and yields unstable intermediate **449**, which upon treatment with acid,[200] or prolonged heating[201] undergoes by aromatization to 3-hydroxypyridines **450** in moderate yields, Figure 40.

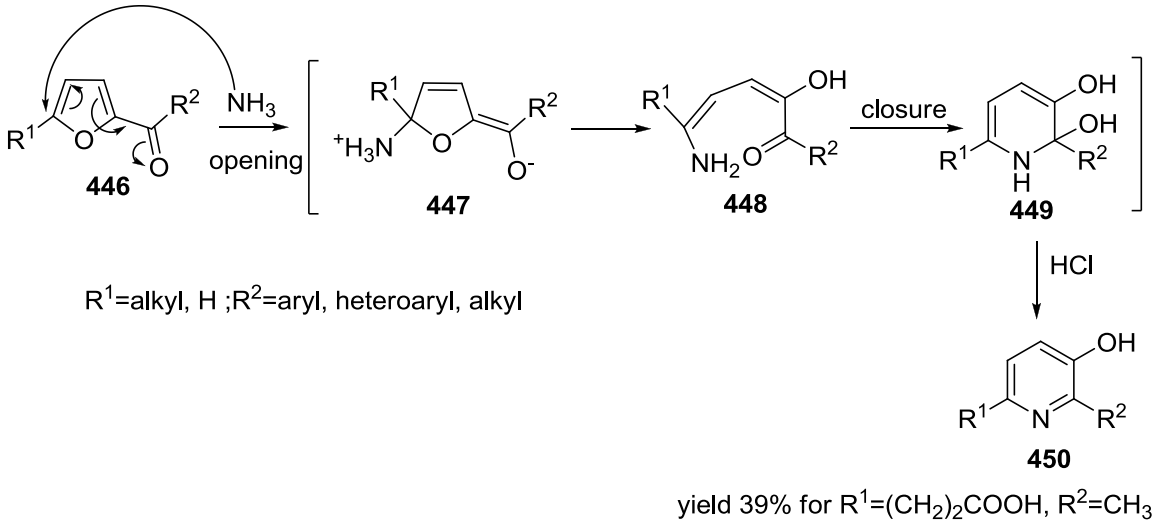
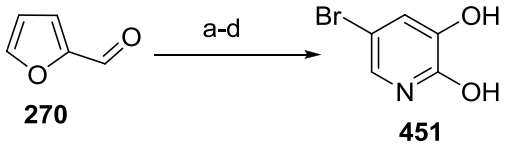


Figure 40. Aminolysis of furan derivatives to hydroxypiridines.

An unusual and a little known example of the oxidative rearrangement of furfural (**270**) to dihydroxypyridine **451** was published by Dallacker,[202] Figure 41. Sequential treatment of furfural with one equivalent of bromine, hydrochloric acid, and then one more equivalent of bromine followed by heating with sulfamic acid led to 5-bromo-2,3-dihydroxypyridine (**451**) in good yield in a one pot-operation.



Reaction conditions: (a) 1 eq. Br_2, H_2O, 0°C; (b) HCl, H_2O, 0°C; (c) 1 eq. Br_2, 0°C; (d) H_2NSO_3H, 50°C, 48%.

Figure 41. Synthesis of 5-bromo-2,3-dihydroxypyridine from furfural.

Six-membered oxygen containing heterocycles such as pyrylium cations, 2- and 4-pyrones also have been successfully used for synthesis of pyridine scaffolds. Different sources of nitrogen can be used for such transformations. Standard aminolysis with methylamine in a sealed tube with methoxy derivative of comenic acid (**452**) led to 4-

pyridone carboxylic acid **453** in excellent yield,[203] Figure 42. Much milder conditions for nitrogen replacement were developed by Kvita,[204] who observed that upon treatment of 2-pyrone **454** with hexamethyldisilazane (HMDS) as the nitrogen source in the presence of a base at room temperature 2-pyridone **455** can be isolated in good yield without the aminolysis of ester group.

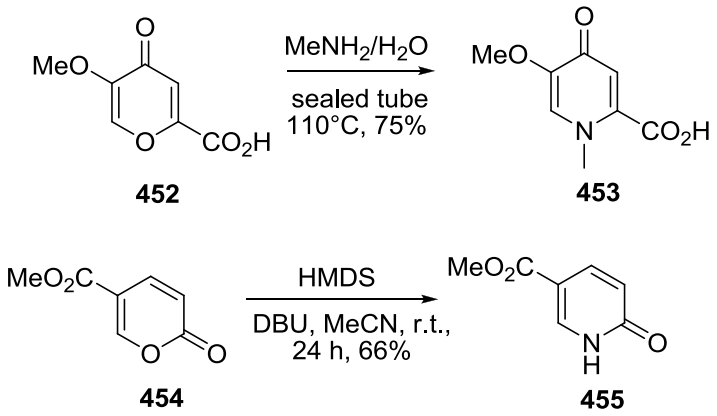


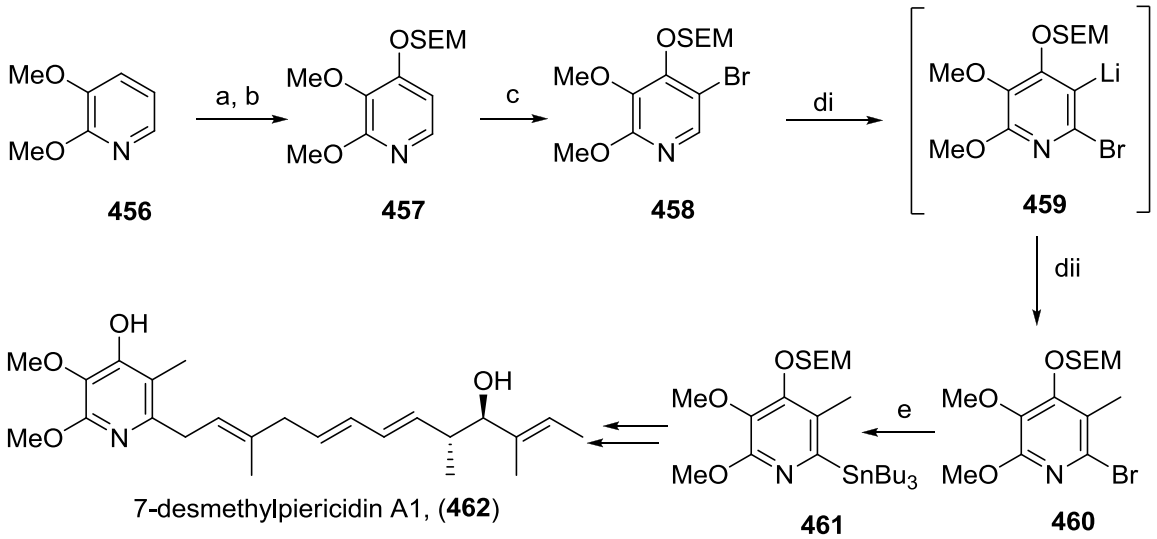
Figure 42. Transformation of 4- and 2-pyrones.

2.3.4. Selected syntheses of pyridine-containing natural products and biologically active compounds.

Natural products containing polyoxygenated pyridine rings constitute a relatively small group of compounds especially in comparison with isoquinoline and indole alkaloids.[146, 205] In the previous chapters quite a large number of diverse and creative methodologies for pyridine synthesis were presented but the ultimate test for every methodology is its application to total synthesis. However, most of the syntheses of polyoxygenated pyridines do not start with the creation of the pyridine, rather a modification of a simple pyridine building block is utilised instead. This occurs due to a large selection of available pyridine building blocks and the widespread application of simple

commercially available pyridines in medicinal chemistry, fine chemistry, and material science. These demands which renders the *de novo* synthesis of pyridine building blocks impractical.

In this chapter the key steps of syntheses of several pyridine-containing natural compounds will be reviewed, which can serve as an inspiration and a guide for the synthesis of analogues of narciclasine. One family of such compounds is the piericidines antibiotics, potent inhibitors of the mitochondrial respiratory chain in eukaryotic and prokaryotic organisms. One approach towards this family was already described in previous chapter, Scheme 45. A completely different strategy was utilized by Keaton and Phillips,[206] in order to provide access to the key pyridine **460**, Scheme 52. 2,3-Dimethoxypiridine (**456**) was submitted to a DoM/borylation/oxidation sequence, followed by protection to provide trialkoxypyridine **457**. Futher DoM provided bromopyridine **458**, which upon treatment with the hindered base lithium 2,2,6,6-tetramethylpiperidide (LTMP) underwent migration of the halogen atom also known as a halogen-dance.[207] Transient aryl lithium intermediate **459** was quenched with methyl iodide to provide **460** which after further metallation and stannylation yielded key fragment **461**. Installation of alkyl side chain by Stille coupling and deprotection yielded 7-desmethylpiericicdine A1 (**462**).

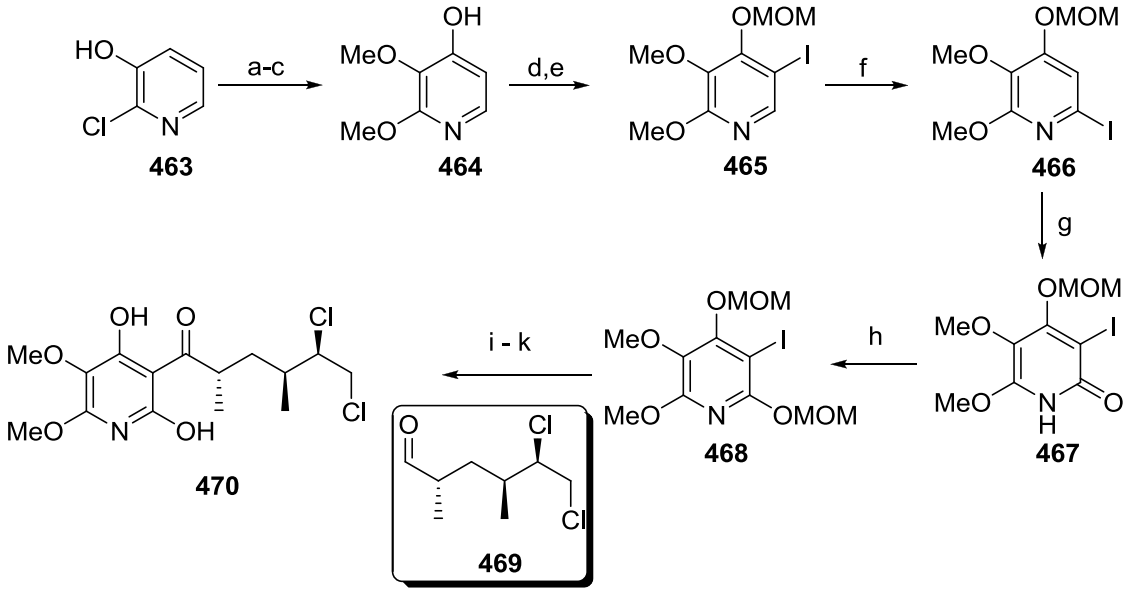


Reaction conditions: (a) (i) *n*-BuLi; (ii) $B(OMe)_3$; (iii) AcOOH, 70%; (b) SEMCl, Ag_2CO_3, 97%; (c) (i) *t*-BuLi, (ii) 1,2-$C_2Br_2Cl_4$ 79%; (d) (i) LTMP, (ii) MeI, 85%; (e) *t*-BuLi, *n*-Bu_3SnCl, 96%.

Scheme 52. Phillip`s synthesis of 7-desmethylpiericidine A1.

Another family of natural compounds with polyoxygenated pyridines are the atpenins. These compounds exhibit their major biological activity as inhibitors of the mitochondrial complex II of the respiratory chain. The syntheses of this family of natural products by different groups share some similar features, especially the halogen dance, so the seminal paper on synthesis of atpenin B by Queginer[208] will be omitted and the most recent synthesis of atpenin A5 by Nakamitsu[209] is described instead. The starting material for this sequence, commercially available 2-chloro-3-hydroxypyridine (**463**) was methylated and treated with sodium methoxide, followed by standard DoM/borylation/oxydation sequence for the introduction of a hydroxyl, Scheme 53. Iodination of 4-hydroxypyridine **464** was followed by protection to attain iodopyridine **465**, which was subjected to a halogen dance reaction in presence of LDA. Product of rearrangement, iodopyridine **466** was treated with the standard *trans*-metallation/borylation/oxidation sequence. It is

interesting to note, that during the oxidation step the by-product, iodine, performed *in situ* halogenation of 2-pyridone intermediate to provide iodopyridone **467**, which was further protected and functionalised by means of *trans*-metallation and coupling with aldehyde **469**. Final oxidation and deprotection yielded atpenin A5 (**470**) in a relatively short sequence.



Reaction conditions: (a) MeI, NaH, DMF 93%; (b) MeOH, NaH, DMF, 86%; (c) (i) *n*-BuLi, (ii) $B(OMe)_3$, (iii) *m*CPBA, 64%; (d) I_2, K_2CO_3, 75%; (e) MOMCl, NaH, DMF, 90%; (f) LDA, 75%; (g) (i) *n*-BuLi, (ii) $B(OMe)_3$, (iii) *m*CPBA, 76%; (h) MOMCl, NaH, DMF, quant; (i) (i) *n*-BuLi, (ii) **469**, 83%; (j) DMP, 86%; (k) TFA, 93%.

Scheme 53. Nagamitsu`s synthesis of atpenin A5.

As can be seen from the two previous examples and some other syntheses of related compounds[210, 211] the halogen dance reaction is a strategic reaction for selective functionalization of pyridine rings and a key step in many total syntheses of natural compounds with polyhydroxypyridine fragments. Together the diverse approaches towards *de novo* synthesis of pyridine core combined with different functionalization tactics present a powerful tool in the design of complex pyridine-containing molecules.

The preceding chapter described previously known approaches towards the synthesis of different *Amaryllidaceae* alkaloids, their structural analogues, the excursion into the field of enzymatic dihydroxylation of arenes, as well as diverse approaches towards syntheses of polysubstituted pyridines. It is hoped that it provided a sufficient overview of the current state of strategies as well as tactics in the field of synthesis of analogues of narciclasine and pacrastistatin and provide the background for the importance of research presented in the next section.

3. Discussion

3.1. Introduction

Natural products have always been an indispensable source and inspiration for new drugs and have produced significant insight into molecular interactions of different biological processes. The Amaryllidaceae alkaloid congeners, in particular the isocarbostyril family, present a good example of a family of bioactive compounds with high activity, a largely unexplored mechanism of action, and a potential as viable drug candidates. Research in this area has been hampered by scarce availability of most of these compounds from natural sources and their poor solubility profiles. While the issue of solubility can be addressed by the synthesis of soluble phosphate prodrugs, the generation of these derivatives still relies on the supply of natural products. Therefore a major goal is to develop a general and divergent approach towards analogues of pancratistatin and narciclasine. In order to perform this task, a few major issues must be addressed. First of all, the synthesis has to provide access to a range of compounds starting from a common starting material. Second, the design of the synthesis needs to be efficient in order to deliver the desired product in the minimum number of steps.

A major theme of the discussion will be focused on our efforts to develop and test a few distinctly different and general approaches to produce analogues of pancratistatin (**2**) and narciclasine (**1**) utilising cyclohexadienediol (**4**) as a common chiral building block. Therefore the discussion is separated into three parts. The first one describes our approach to C-1 homologues of pancratistatin and synthesis of three new compounds (**5a-**

c), Figure 43. This approach is based on a route previously developed in our group towards C-1 analogues of 7-deoxypancratistatin.[78, 79] Application of a similar strategy, differences and modifications on the way to the three novel analogues and their anticancer activity are described.

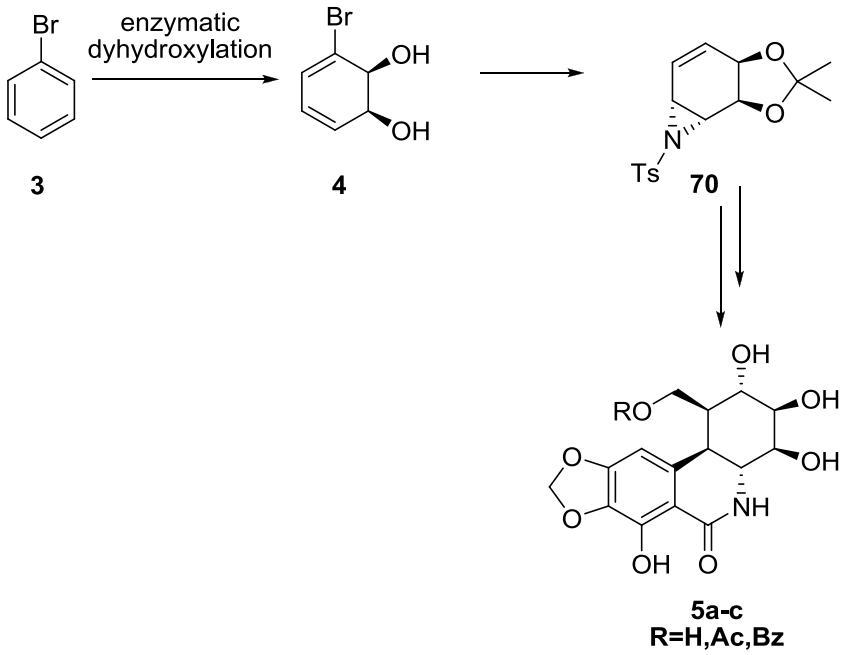


Figure 43. Route towards C-1 homologues of pancratistatin.

The second part is focused on the approach towards pyridine analogues of pancratistatin (**472**) based on the cobalt-catalyzed [2+2+2] cycloaddition reaction. Our investigation of this reaction and attempts to produce and further functionalize analogues based on the skeleton of **472** is discussed.

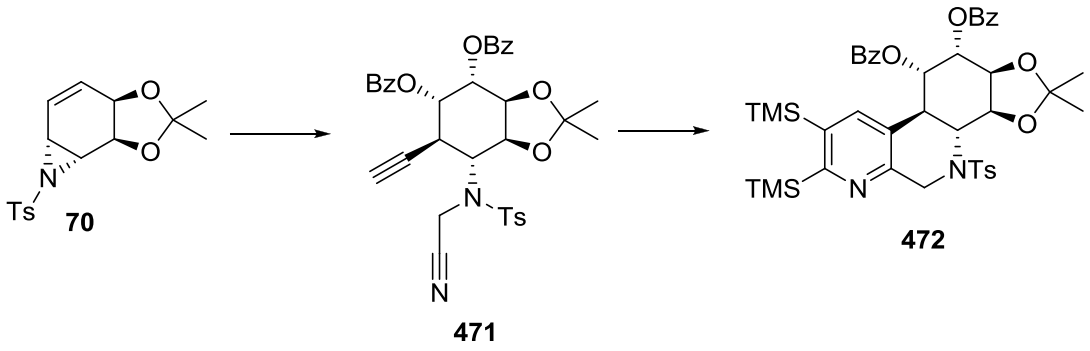


Figure 44. [2+2+2] cycloaddition approach towards A-pyridine ring analogues.

In the last section a convergent synthesis of different aza-analogues (**6**, **476**, **7**) of narciclasine is described, based on the intramolecular Heck coupling of amides **475** and **478**. These amides were in turn produced from halopyridine carboxylic acids **474** and **475** and the protected conduramine **473**. The main focus of this section will be an development of conditions for the Heck reaction and the synthesis of pyridine building blocks.

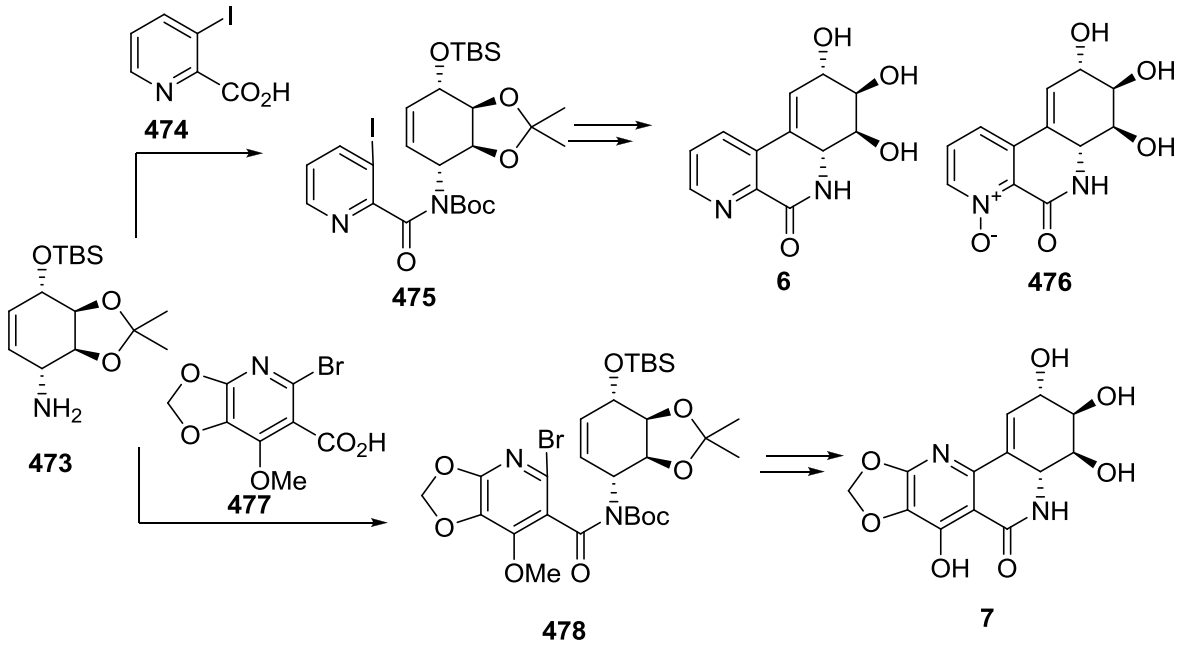


Figure 45. Intramolecular Heck approach to heterocyclic narciclasine analogues.

The biological activity of these derviatives as well as new information on the minimal pharmacophore requirements will be discussed as well as the beneficial effect of certain functional groups and new directions for analogues development.

3.2. Synthesis of C-1 analogues of pancratistatin

The Hudlický group has dedicated significant efforts towards the synthesis of Amaryllidaceae constituents and their unnatural analogues for the past 20 years. Recently the group published a series of papers on the synthesis and biological activity of the C-1 homologues of 7-deoxypancratistatin.[78, 79] Two of these compounds, namely the C-1 hydroxyl (**183a**) and C-1 acetate (**183b**) have shown activity against a variety of cancer cell lines comparable to those of the natural congener. It is known that the 7-hydroxy group plays an important role in the cytotoxicity as Amaryllidaceae isocarbostyril congeners with this substituent present are 10-100 times more active towards different

cancer cell lines. The goal of our project therefore was to explore and utilize a similar synthetic strategy for the synthesis of a few new analogues of pancratistatin and study the influence of different substituents in the position C-1 on the anti-cancer activity.

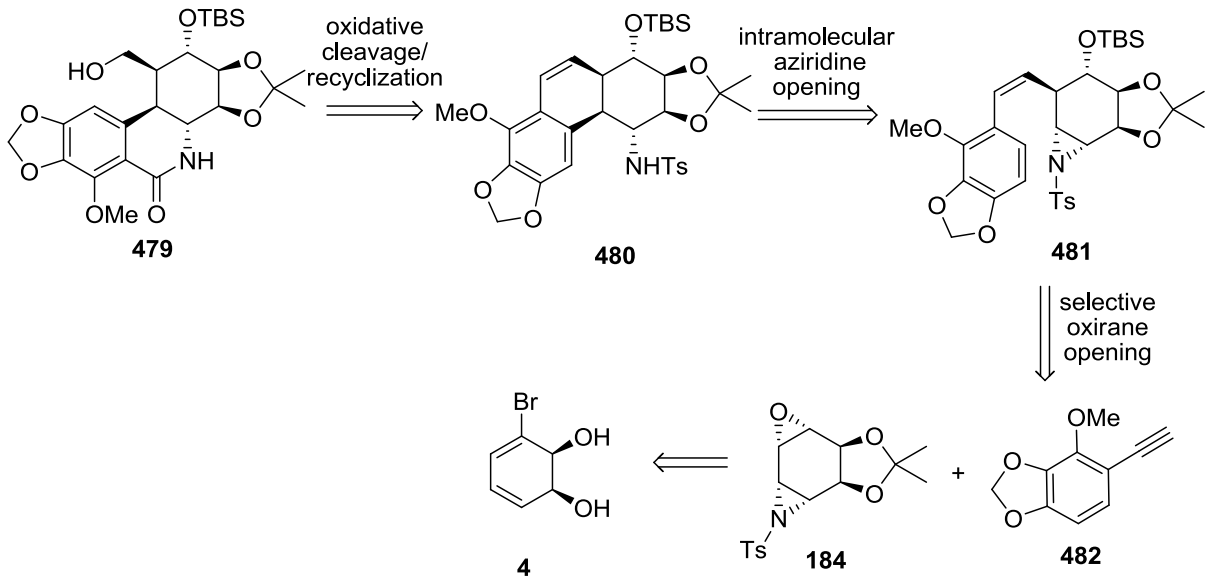
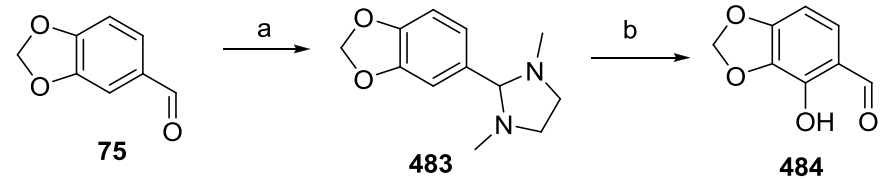


Figure 46. General retrosynthetic strategy towards C-1 homologues.

The general strategy of the synthesis is outlined in Figure 46. In order to study different substituents on the C-1 position, alcohol **479** would present an ideal common intermediate. It could be generated from phenanthrene **480** *via* an oxidative cleavage/oxidative recyclization sequence. Intramolecular opening of aziridine **481** would serve as a good route towards functionalized phenanthrene **479**. Generation of this aziridine **481** was envisioned through selective opening of oxirane **175** by alkyne **482**. The chiral cyclohexadiene diol **4** which bears all necessary functionality to produce previously reported oxirane **175**[78] and was envisioned as a good starting material for the synthesis.

The synthesis of intermediate **481** began with the production of the first key intermediate, alkyne **482**. The shortest and the most convenient way to approach this compound was to start from readily available piperonal (**75**). Introduction of the hydroxyl group was envisioned *via* DoM/borylation/oxidation sequence. Several of the total syntheses of pancratistatin and narciclasine have relied on this strategy to introduce the required 7-hydroxyl group.[27, 42, 52] Most of these transformations were performed with tertiary amides of piperonylic acid, which were proven to be cumbersome to reduce to the corresponding aldehydes. In order to shorten the synthetic sequence a directing group was sought, which could be deprotected directly to produce aldehyde under mild conditions. Two different directing group have been described in the literature for direct *ortho*-metalation of piperonal: 1,3-dimethylimidazolidine **483**[212] and cyclohexylimine **485**.[213]



Reaction conditions: (a) N,N'-Dimethylethylenediamine. toluene, reflux, 79%; (b) (i) RLi, additive, solvent; (ii) $B(OMe)_3$, (iii) AcOH, H_2O_2.

Scheme 54. Studies of DoM of 1,3-dimethylimidazolidine.

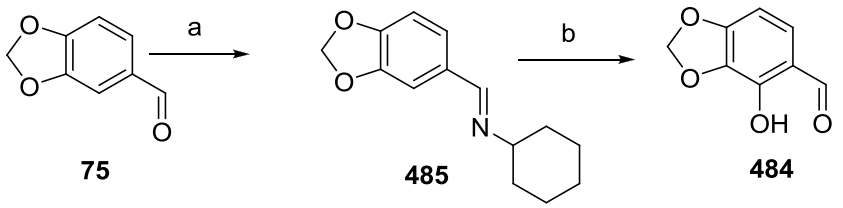
In our hands, intermediate **483** failed to perform the transformation in the previously reported yields, Scheme 54. Different conditions and organometallic reagents for the metallation were tried and these attempts are summarized in Table 5. Aside from the fact that the yields of the desired transformation were low, also the synthesis of intermediate **483** itself was proven to be cumbersome. The original paper reported distillation of

product at low pressure (108 °C/5*10^{-3} Torr); we performed column chromatography, which proved to be impractical on a large scale.

Table 5. *Ortho*-metallation conditions.

Conditions,solvent	RLi; eq.; (additive)	Yield,%
−78 °C→r.t. → −78 °C, Et_2O (lit. procedure)	*t*-BuLi; 2;	30
−78 °C →−10 °C, THF	*t*-BuLi; 2	22
−78 °C →0 °C →−78 °C, Et_2O	*t*-BuLi; 1.2	20
−78 °C →r.t., Et_2O	*n*-BuLi; 1.2 (TMEDA)	26
−78 °C →r.t., Et_2O	*n*-BuLi; 2 (TMEDA)	34
−78 °C →r.t., THF	*n*-BuLi; 1.2(TMEDA)	30
−78 °C →r.t., THF	*n*-BuLi; 2(TMEDA)	39

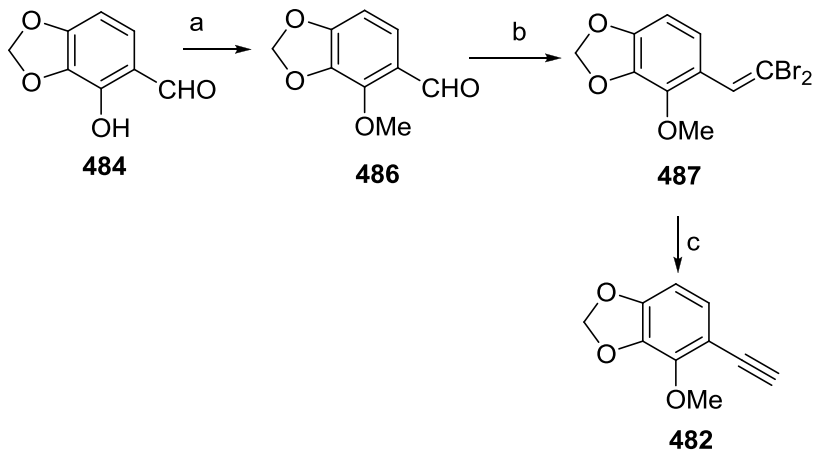
We tested therefore a second approach with the introduction of a cyclohexylimine auxiliary **485**, Scheme 55. Isolation of intermediate **485** did not pose any problems, since it was a solid and was easily recrystallized from methanol. *ortho*-Metallation was performed at -78 °C, and after borylation, oxidation and hydrolysis provided the desired hydroxyladehyde **484**.



Reaction conditions: (a) Cyclohexylamine, 85%; (b) (i) *n*BuLi, THF, -78°C (ii) $B(OMe)_3$, (iii) AcOH, H_2O_2; (iv) 6M HCl, reflux, 71%.

Scheme 55. *Ortho*-metallation of cyclohexylimine **485.**

Methylation of phenol **484** was performed with dimethyl sulfate in the presence of potassium carbonate, yielding methoxyaldehyde **486**, Scheme 56. This compound was allowed to react with carbon tetrabromide in the presence of triphenylphosphine to provide dibromoalkene **487**, which after treatment with *n*BuLi provided desired alkyne **482** in 5 steps from piperonal in good overall yield. The largest scale, this sequence was performed on, provided 40 g of alkyne in a single run.

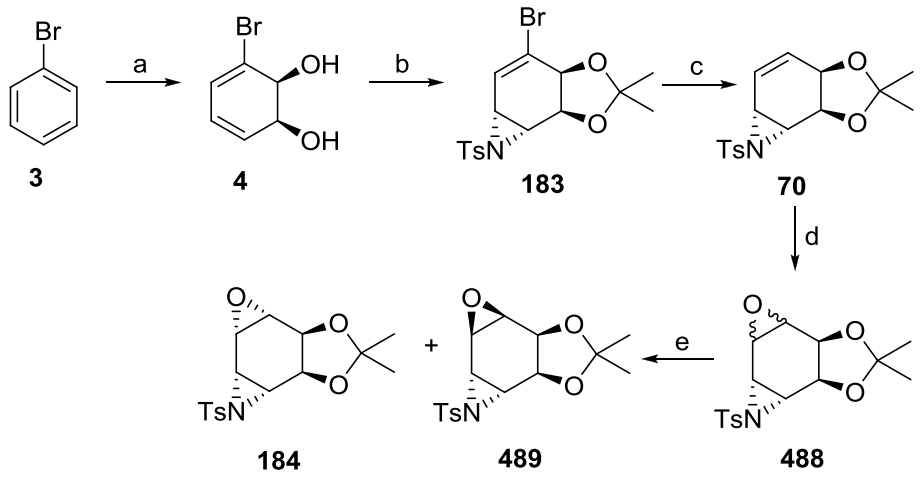


Reaction conditions: (a) Me_2SO_4, K_2CO_3, acetone, reflux, 85%; (b) CBr_4, PPh_3, CH_2Cl_2, 79%; (c) *n*BuLi, THF, -78 °C, 82%.

Scheme 56. Synthesis of alkyne **482**.

The chiral epoxyaziridine **175** was prepared by a previously described route developed in the Hudlický group.[78] The product of enzymatic dihydroxylation of bromobenzene (**3**),

the chiral diol **4**, was protected as acetonide **183** and immediately subjected to aziridination protocol under the Yamada-Evans conditions,[80, 81] Scheme 57. Complete facial selectivity was achieved as a result of the steric hindrance of the ketal. The bromine atom was removed under radical reduction conditions with tri-*n*-butyl tin hydride to provide *N*-tosyl aziridine **70** in good yield. Epoxidation of the alkene moiety was performed by treatment with *m*-chloroperbenzoic acid in refluxing dichloroethane. This epoxidation did not provide complete facial selectivity, but instead led to a 3:1 mixture of diastereomers in favour of the desired isomer **184**. This ratio was increased by three successive fractional recrystallizations from isopropanol to 6:1-7:1 and produced 10 g of epoxyaziridine **184** in a single run.



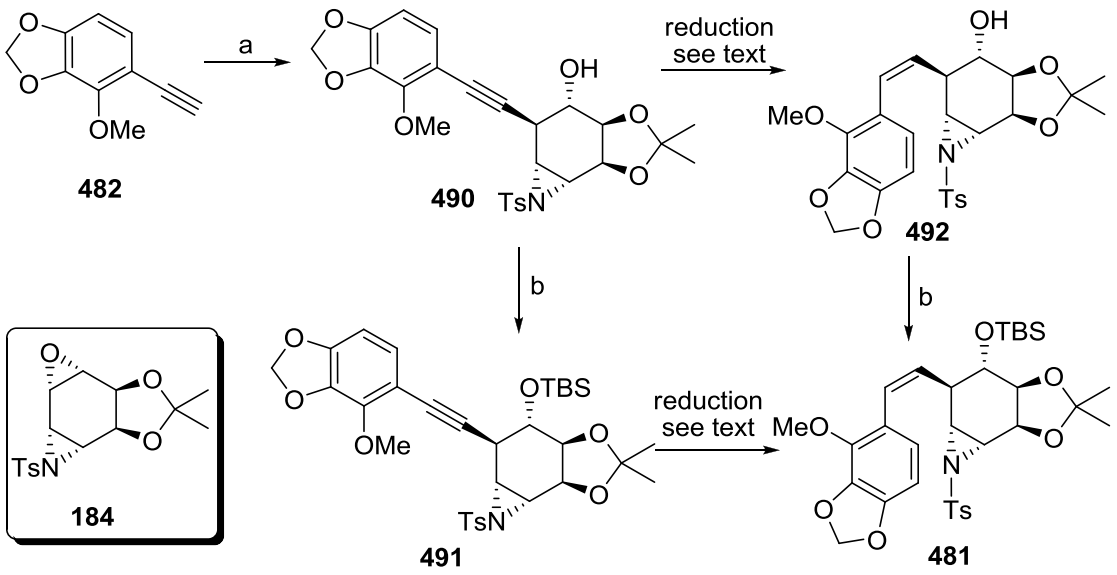
Reaction conditions: (a) *E. coli* JM109 (pDTG601A), 10-15 g/L; (b) (i) 2,2-DMP, *p*-TsOH, acetone; (ii) PhI=NTs, $Cu(acac)_2$, 0 °C to r.t., 52%; (c) nBu_3SnH,THF, AIBN, reflux, 76%; (d) *m*CPBA, 1,2-DCE, reflux, 95% *dr* 3:1; (e) fractional recrystallisation from *i*PrOH.

Scheme 57. Synthesis of chiral epoxiaziridine **184**.

The next step consisted of selective nucleophilic opening of oxirane **184** by the alkynylalane formed *in situ* from the anion dervided from alkyne **482** and

dimethylaluminum chloride, Scheme 58. Stringent reaction conditions and carefully controlled temperature had to be used to ensure selective opening of oxirane moiety in the presence of the aziridine ring. The alkyne **490** was somewhat unstable and the yield was significantly increased (from 42 to 70%) if the crude reaction mixture is directly submitted to the next protection step with *t*-butyldimethylsilyl triflate.

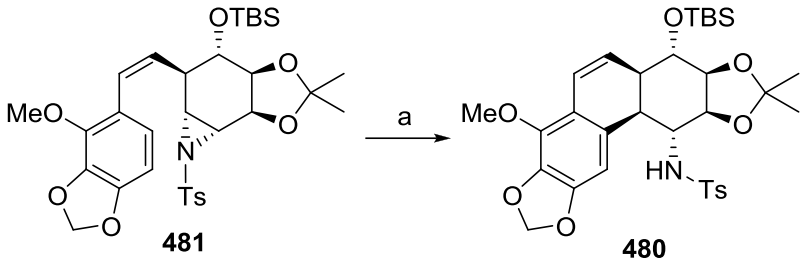
The next step was to perform selective reduction of alkyne **490** to the *Z*-alkene **481**. The original conditions for the reduction of 7-deoxyanalogues[78] consisted of reduction with boranes. Unfortunately, reaction of dicyclohexyl borane with alkyne **491** did not lead to the desired product, probably because of the steric hindrance of the methoxy group. Catalytic hydrogenation is one of the most common ways to generate Z-alkenes;[214] we discovered that this particular reaction was sensitive to the steric environment of the triple bond, *i.e.* reduction of the TBS-protected alcohol **491** did not lead to reproducible yields. Much more successful results were observed during the reduction of alcohol **492**. Nevertheless, over-reduction of the alkyne posed a significant problem and screening of conditions and solvents for selective reduction was performed.



Reaction conditions: (a) (i) *n*BuLi, PhMe, -50 °C; (ii) Me_2AlCl, -40°C to r.t.; (iii) **184**, -30 °C to r.t., 42%; (b) TBSOTf, Et_3N, CH_2Cl_2, 97% for **481**; 70% for two steps for **491**.

Scheme 58. Selective opening of epoxide and further reduction.

Lindlar catalyst and palladium on carbon in methanol led to significant over-reduction. Reduction of substrate in the ethyl acetate did not proceed well, but it was discovered that addition of 20 mol. % of quinoline to 20 mol. % Lindlar catalyst and reduction in methanol allowed for selective reduction and nearly quantitative conversion with negligible amounts of by-products was observed. After protection with a *tert*-butyldimethylsilyl group the key intermediate **481** was submitted to silica-gel catalysed closure, Figure 47. This step consisted of adsorption of compound on pre-dried silica-gel and heating at 120 °C for 24-36 h. It was discovered that traces of quinoline (*ca.* 5%) from the reduction step led to a significant increase in yield, albeit with a prolonged reaction time. Our hypothesis for this phenomenon is that quinoline neutralises inherent acidity of silica-gel and therefore reduces unwanted decomposition of the acid-labile groups in compound **481**.



Reaction conditions: (a) SiO_2, 120 °C, 36h, 74%

Figure 47. Solid phase silica-gel-catalyzed closure.

After securing access to phenanthrene **480**, our next goal was to perform oxidative cleavage of the olefin bond which upon oxidative cyclization, was expected to form hemiaminal **494**, Figure 48. The original conditions developed for 7-deoxypancratistatin analogues for cleavage of this type of bonds (OsO_4/IO_4^-) did not lead to a clean transformation and therefore different conditions were screened.

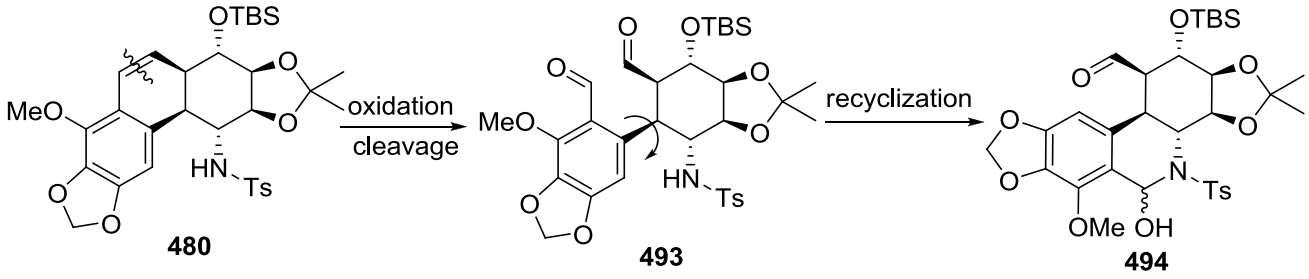
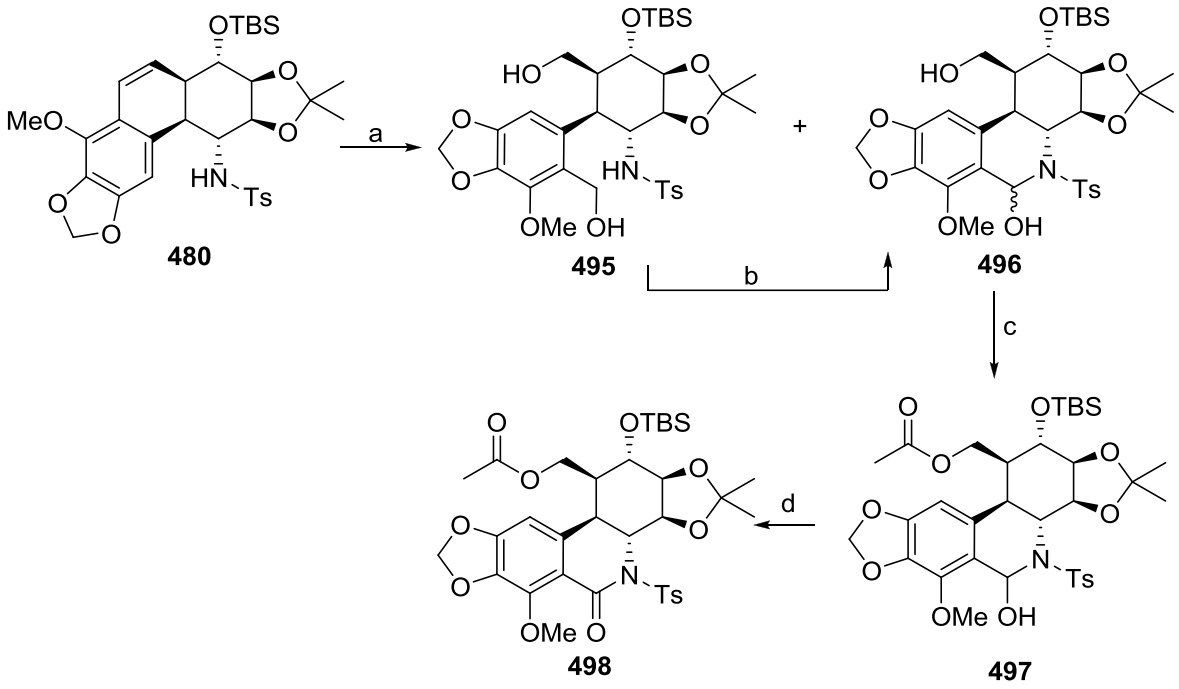


Figure 48. Oxidative cleavage and recyclization of phenathrene **480**.

Ozonolysis is one of the most convenient ways to cleave double bonds. But an important issue is the selectivity of double bond oxidation using ozone. Electron–rich aromatic rings are prone to oxidation by ozone and therefore standard conditions of ozonolysis, *i.e.* the appearance of a blue colour of excess of ozone in solution, are too destructive for the selective transformation. Therefore, selective indicator such as Sudan Red 7b[215] was

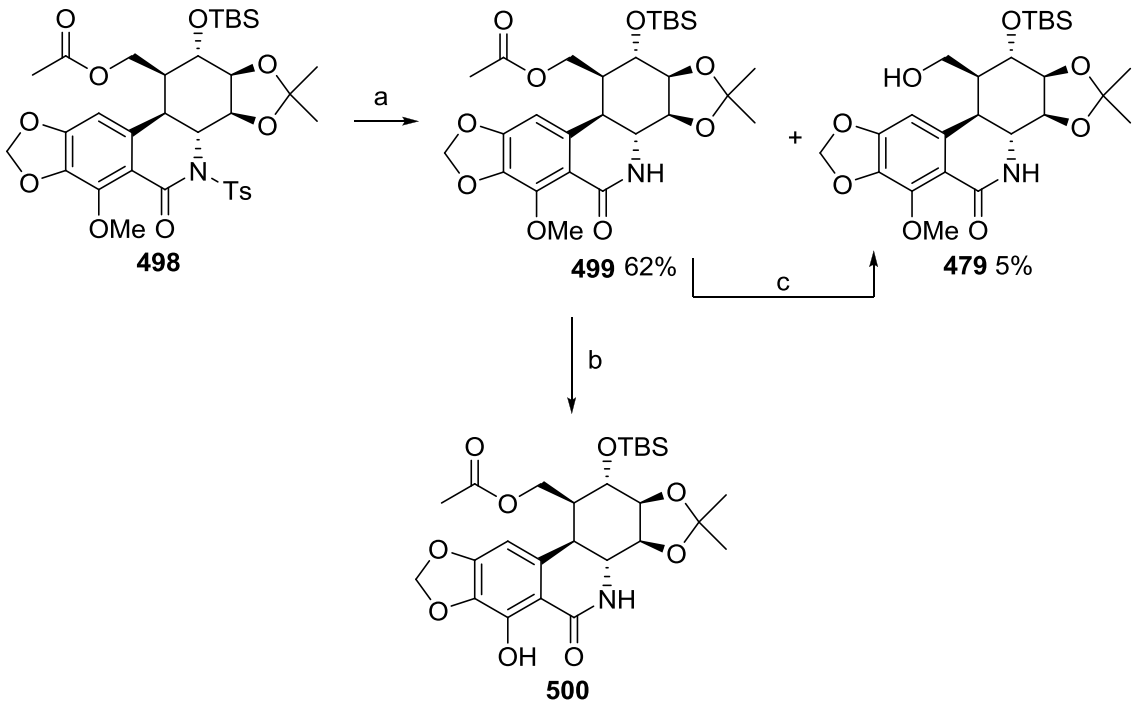
introduced as an indicator to ensure selective cleavage of the alkene. Reductive quenching was performed with sodium borohydride to provide a mixture of diol **495** and hemiaminal **496** in 2:1 ratio, Scheme 59. It was found that this particular reaction turned out to be sensitive to the solvent; upon performing the reaction in methanol significant decomposition to polar by-products was observed. Transformation of diol **495** to hemiaminal **496** was investigated next. Screening of different conditions for selective benzylic oxidation was performed. The only conditions that led to good conversion to hemiaminal **496** were the use manganese dioxide in dichloromethane. The next goal was to oxidize the heminaminal moiety to an amide to complete the skeleton of the desired target. It required selective protection of the primary alcohol in **496** in the presence of the hemiaminal, which was achieved by reaction with acetic anhydride with pyridine in dichloromethane and yielded a mixture of acetate protected anomeric hemiaminals **497** in 87% yield. Different oxidation conditions were tried, including IBX, Dess-Marin periodinane, PDC, but the best yield was obtained with the milder oxidation conditions of tetrapropyl ammonium perruthenate and *N*-morpholine oxide, also known as the Ley-Griffith oxidation.[216] Desired amide **498**, the common intermediate for the synthesis of all C-1 homologues of pancratistatin, was isolated with 84% yield.



Reaction conditions: (a) (i) O_3, Sudan red 7b; (ii) $NaBH_4$, 60% of **495**, 31% of **496**; (b) MnO_2, CH_2Cl_2, 87%; (c) Ac_2O, pyridine, CH_2Cl_2, 87%; (d) TPAP, NMO, CH_2Cl_2, 84%.

Scheme 59. Conversion of phenanthrene **480** to amide **498**.

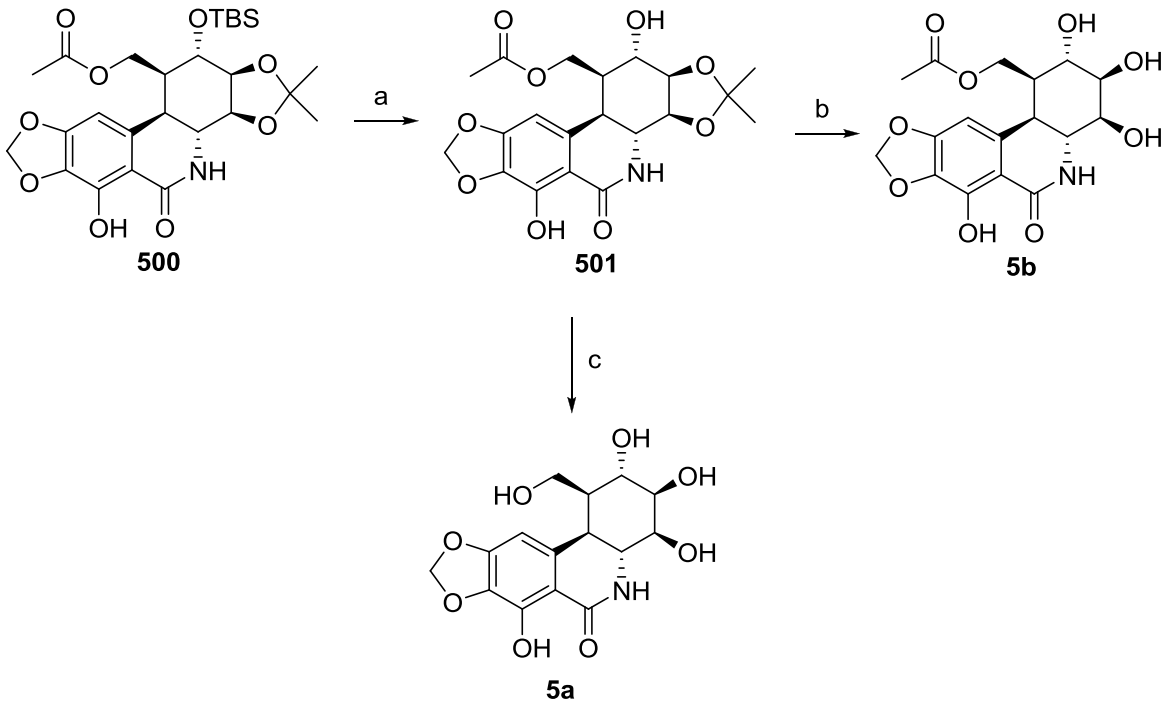
Having secured access to this fully protected C-1 acetoxymethyl pancratistatin, deprotection steps were studied. Detosylation of **498** was performed by means of reductive cleavage with sodium napthalenide to provide amide **499** in 62% yield, alcohol by-product **479** was also isolated in small quantities from this reaction, Scheme 60. Screening of standard demethylation conditions[217] were performed, including sodium thiolate,[218] boron tribromide[52] and lithium chloride;[25] only the latter conditions provided the desired phenol **500** successfully. Deprotection of the acetate group in **499** was also studied in order to provide access to a different ester substituent on position C-1. Removal of acetate group proceeded smoothly upon treatment of **499** with a concentrated aqueous solution of sodium hydroxide in methanol.



Reaction conditions: (a) Na, naphthalene, DME, -78°C; (b) LiCl, DMF, 120°C, 67%; (c) KOH, H_2O, MeOH, 80%.

Scheme 60. Production of common intermediate **479.**

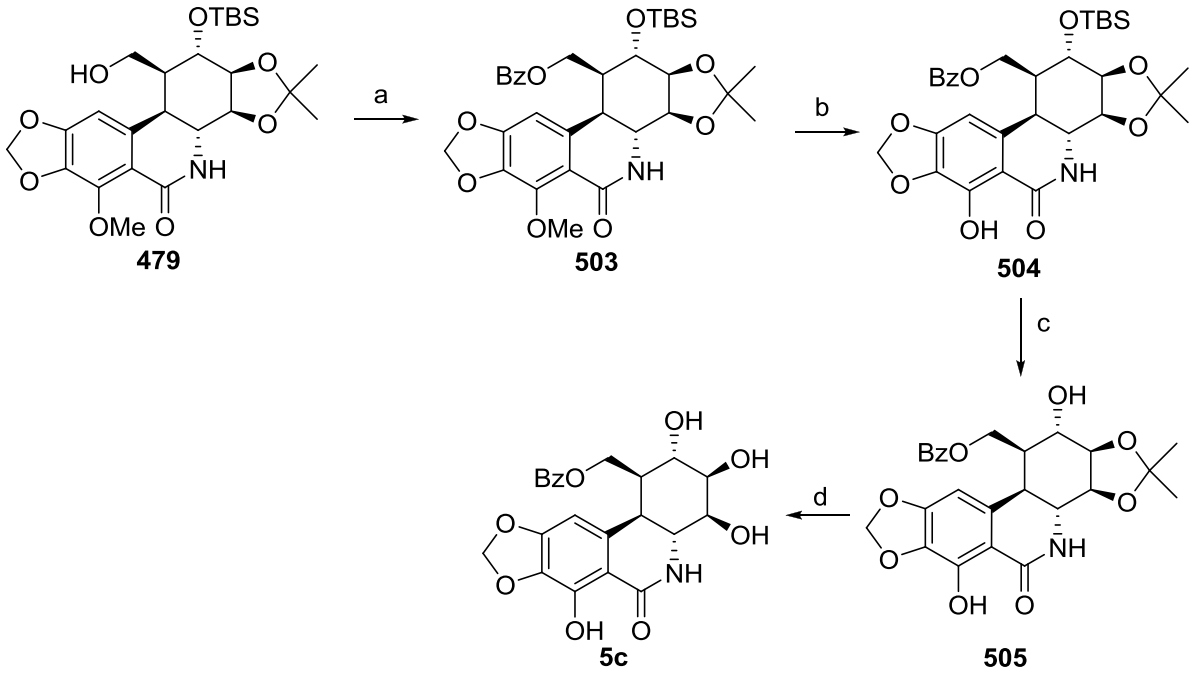
Removal of the silyl group did not pose any problems and was performed under standard conditions with tetrabutyl ammonium fluoride (TBAF). The last step, however, posed some difficulties, since the previously reported conditions[79] for selective deprotection of acetonide led to deprotection of the acetate as well and formation of C-1 pancratistatin homologue **5a**, Scheme 61. Treatment of acetonide **501** with trifluoroacetic acid at 0 °C provided C-1 acetate **5b** in excellent yield.



Reaction conditions: (a) TBAF, THF, 0 °C, 95%; (b) 3% HCl, MeOH, 92%; (c) TFA, CH_2Cl_2, 90%.

Scheme 61. Deprotection sequence towards C-1 pancratistatin homologue **5a** and acetate **5b**.

The next goal was to produce the benzoate ester of C-1 pancratistatin homologue, since it has been known that this particular group plays a beneficial role in anticancer activity of Amaryllidaceae congeners. Benzoylation of alcohol **479** was performed with benzoyl chloride and provided ester **503**, which was submitted to deprotection conditions, similar to those employed before for C-1 acetate **5a**, to yield C-1 benzoate **5c** in three steps, Scheme 62. Synthesis of the three analogues was performed with 17 steps in the longest linear sequence. Biological studies of these three analogues **5a**, **5b**, **5c** were performed on a panel of cancer cell lines and will be discussed in Section 3.5.



Reaction conditions: (a) BzCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, 64%; (b) LiCl, DMF, 120 °C, 83%; (c) TFA, CH_2Cl_2, 90%.

Scheme 62. Synthesis of benzoyl acetate **5c**.

3.3. [2+2+2] Cycloaddition approach to pyridine analogues of pancratistatin

After performing the synthesis and anticancer evaluation of the C-1 analogues, our attention was drawn towards the synthesis of much less explored analogues of the A-ring of pancratistatin. Since it is common practice to utilise a pyridine fragment as a bioisostere of a benzene ring for improving pharmacokinetics,[219] we decided to design a general route towards pyridine analogues of pancratistatin. Our attention was drawn to a [2+2+2] cycloaddition strategy[167-169] of pyridine ring formation, since it offers general

versatility and has been shown to work successfully in the synthesis of similar *bis*-silylated analogues of pancratistatin.[94, 95]

The general retrosynthetic scheme is outlined on Figure 49. *bis*-Silylated pyridine **472** was considered the most convenient intermediate to provide access to polyoxygenated pyridine analogue **506** through oxidation reactions. Synthesis of the key heterocyclic intermediate **472** was envisioned *via* a cobalt-catalyzed [2+2+2] intermolecular cycloaddition between *bis*(trimethylsilyl)acetylene (**269**) and nitrile **471**. This cyanoalkyne **471** in turn can be attained by functionalization of aziridine **70** by means of nucleophilic opening, selective dihydroxylation of the double bond, and alkylation of the amide with the nitrile fragment. Aziridine **70** is a common intermediate in the synthesis of Amaryllidaceae alkaloids in the Hudlický group and was generated in three steps from cyclohexadiene diol **4**.

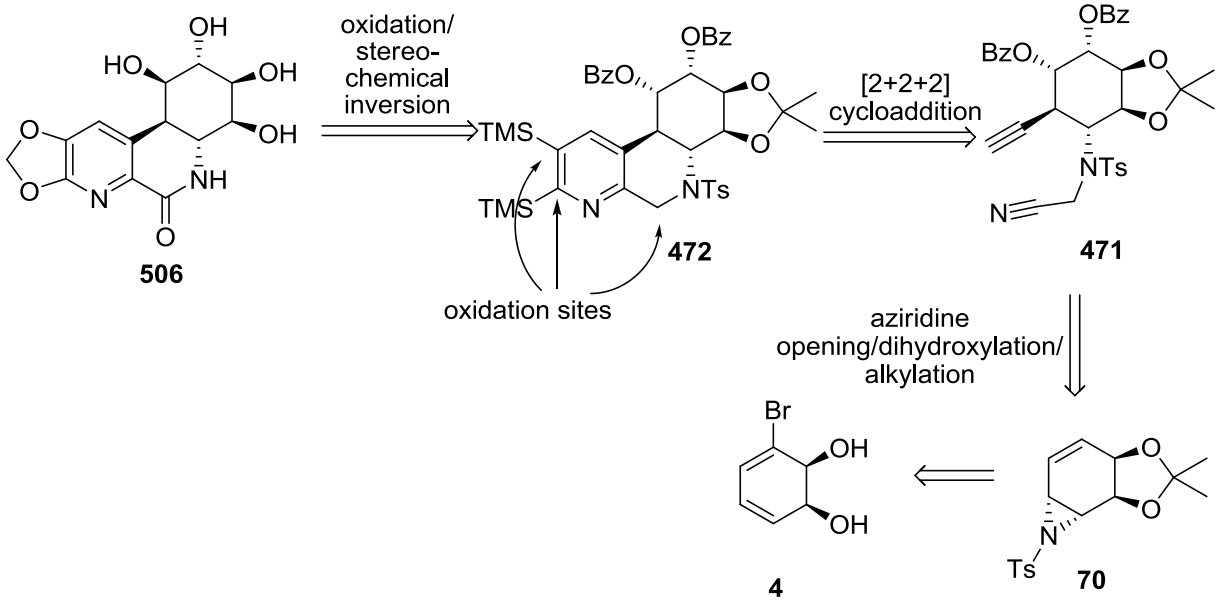
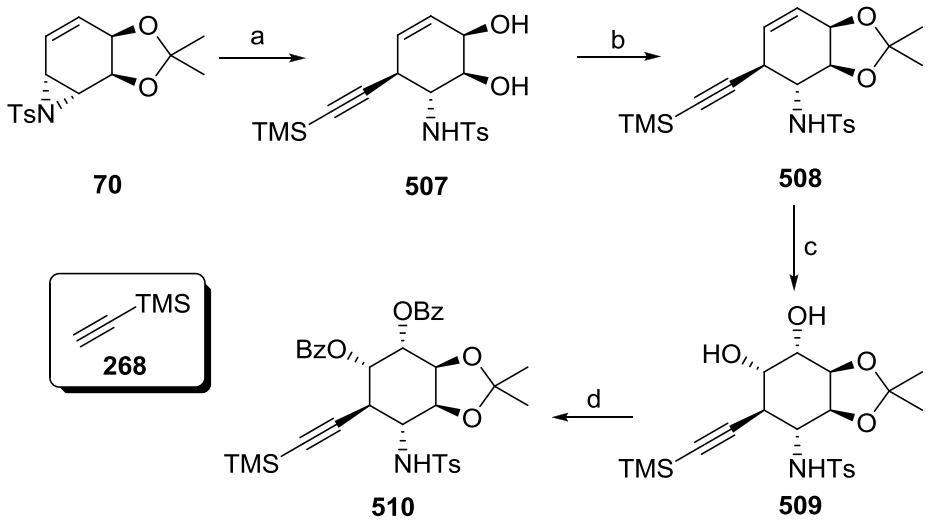


Figure 49. Retrosynthetic scheme for the [2+2+2] strategy of pyridine ring analogues.

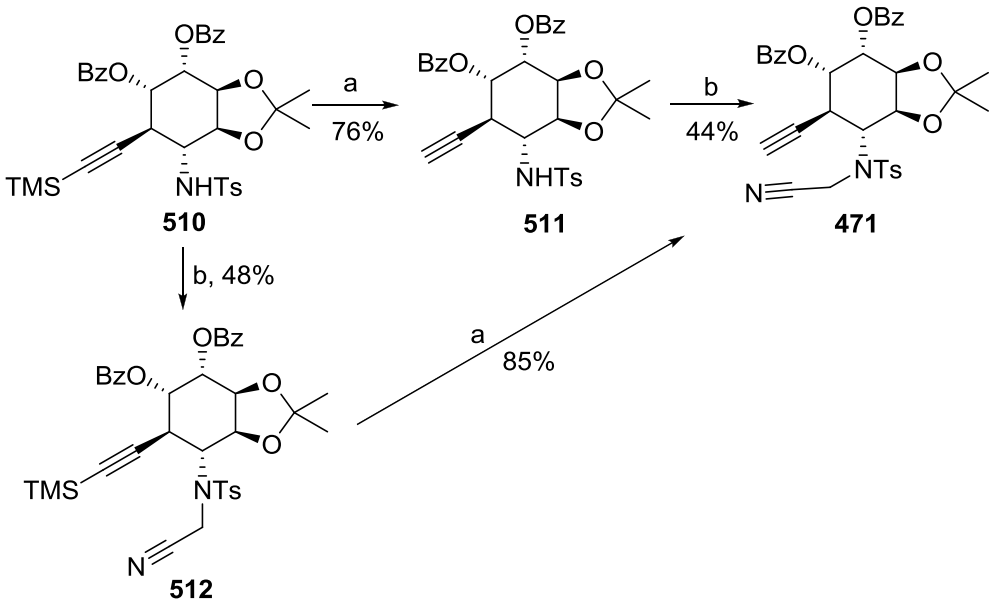
Synthesis of aziridine **70** was discussed in the previous Section 3.2, Scheme 57. Aziridine **70** was submitted to a protocol previously developed in the Hudlický group[92] of nucleophilic opening of the aziridine moiety with a large excess of alkynylalane generated *in situ* from lithium trimethylsilyl acetylide and aluminium chloride, Scheme 63. Simultaneously, the acetonide was hydrolyzed during this reaction, which required reprotection to provide tosylamide **508** in good yield. Selective dihydroxylation of the alkene fragment in the presence of the alkyne was performed by treatment with osmium tetroxide in presence of *N*-methylmorpholine *N*-oxide. This reaction was performed in dichloromethane, unlike the usual conditions,[220] which involve polar solvent system. These were the only conditions that provided a reasonable conversion without significant decomposition of the product **509**. Benzoylation of diol **509** provided protected alkyne **510** in good yield.



Reaction conditions: (a) (i) **268**, *n*BuLi, PhMe, -50 °C; (ii) $AlCl_3$, PhMe, -50 to 0°C; (iii) HCl, H_2O; (b) 2,2-DMP, TsOH, 69% for two steps; (c) OsO_4, NMO, CH_2Cl_2, 45% (77% brsm); (d) BzCl , pyridine, 0 °C, 80%.

Scheme 63. Synthesis of alkyne **510**.

Our next goal was to study the alkylation of the amide fragment in tosylamide **510** to yield α,ω-cyanoalkyne **471**, Scheme 64. Conditions originally developed on the Hudlický group[94, 221] for the synthesis of dialkyne **264** consisted of desilylation with a non-basic reagent, tetrabutyl ammonium triphenyldifluorosilicate (TBAT), followed by alkylation. In this particular case two different routes were studied: (i) desilylation of amide **510** followed by alkylation with chloroacetonitrile in the presence of sodium *bis*(trimethylsilyl)amide or (ii) alkylation of amide **510** with chloroacetonitrile followed by desilylation of formed α,ω-cyanoalkyne **512**. The second route was shown to be more efficient in terms of cleaner conversion, and overall yield.

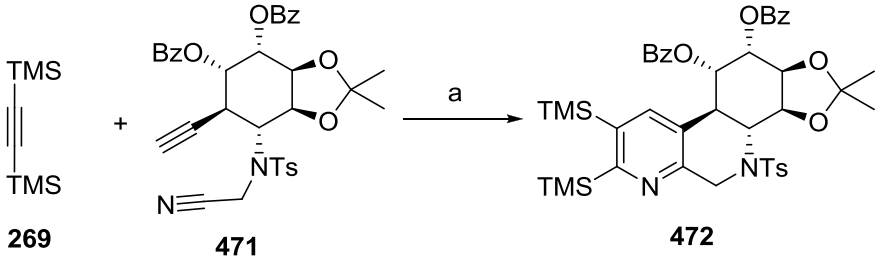


Reaction conditions: (a) TBAT, MeCN, 76%; (b) $ClCH_2CN$, NaHMDS, nBu_4NI, THF, -70 to 0°C, 44%;

Scheme 64. Synthesis of key intermediate **471.**

Having secured the access to the key intermediate α,ω-cyanoalkyne **471**, the cobalt-catalyzed cycloaddition with *bis*-trimethylsilylacetylene (**269**) was studied, Scheme 65. In order to prevent side-reactions, such as the unproductive cycloaddition of two molecules

of **471** and decomposition of the labile precatalyst $CpCo(CO)_2$ the reaction was performed by slow addition of the solution of **269**, **471** and $CpCo(CO)_2$ to the refluxed solution of **269** and $CpCo(CO)_2$ in xylenes under irradiation with visible light. Syringe pump addition over long periods of time (36 h), as was used in the previous approaches,[94, 221] did not lead to better results.



Reaction conditions: (a) $CpCo(CO)_2$, xylenes, hν, reflux, 55%.

Scheme 65. Synthesis of pyridine skeleton via [2+2+2] cycloaddition.

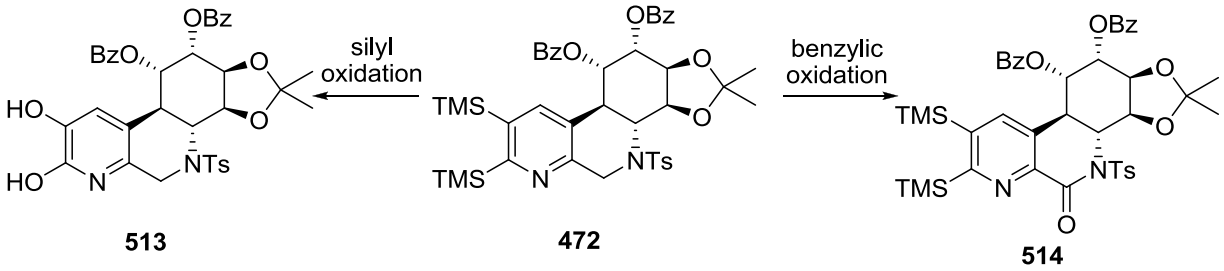
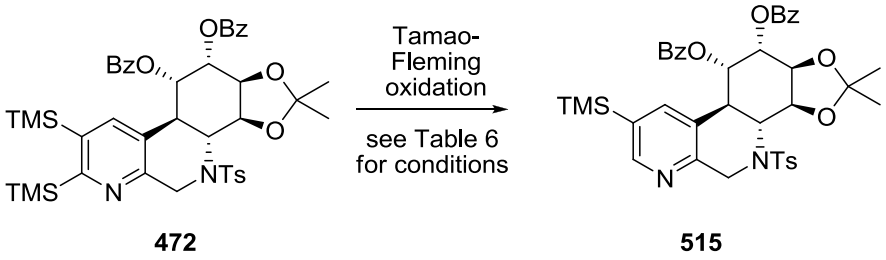


Figure 50. Two required oxidation transformations of **472**.

In order to install the functional groups required to mimic pancratistatin (**2**) in the tricyclic pyridine **472** two different oxidations were required to occur. First of all, replacement of the silyl groups by hydroxyls was envisioned. This approach was based on the fact that silicon group can serve as a masked oxygen and can be replaced in the well-known Tamao-Fleming oxidation.[222] A variety of different conditions was attempted, see Table 6, but unfortunately these conditions did not lead to the formation of

the desired product **513**, the only isolated products were either remaining unreactive starting material or formation of protiodesilylated pyridine **515** (by ^{1}H NMR), Figure 51.



472 **515**

Figure 51. Tamao-Fleming oxidation of *bis*-silylated pyridine **472.**

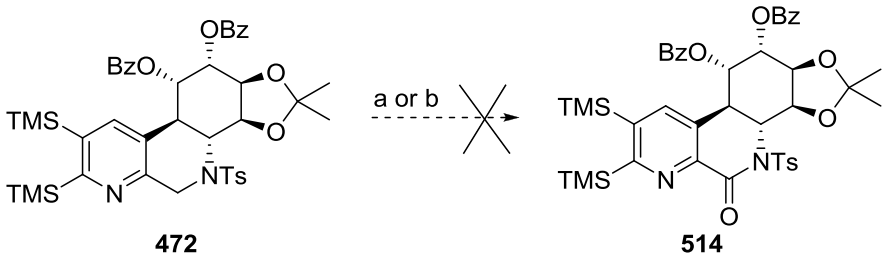
It has also been observed that upon prolonged exposure to air of sample pyridine **472** the same by-product **515** started to form. All of these observation and some precedents from the literature[223] on silylated pyridines led to the conclusion that the trimethylsilyl group is not a viable group for performing the transformation to the hydroxyl functionality.

Table 6. Conditions for the oxidations of silyl groups (Tamao-Fleming reaction).

Reagents	Solvent	Product
KF, H_2O_2, $NaHCO_3$	THF	**472** (SM)
KF, H_2O_2, $NaHCO_3$	THF/MeOH	**515**
KF*HF, H_2O_2	MeOH	**515**
KF*HF, H_2O_2, $NaHCO_3$	MeOH	**515**

Further studies on the model pyridine **472** were performed in order to establish general conditions for benzylic oxidation. The first conditions attempted were those previously

established in the Hudlický group for a similar system:[93, 216] with catalytic ruthenium trichloride and sodium periodate in biphasic solvent mixture, Figure 52. Unfortunately no reaction occurred under this conditions. The second approach was based on well-known[224] selenium dioxide oxidation of benzylic positions. But upon prolonged reflux with SeO_2 in ethyl acetate only monodesilated pyridine **515** was isolated.



Reaction conditions: (a) $RuCl_3$, $NaIO_4$, CCl_4/MeCN/H_2O; (b) SeO_2, EtOAc, reflux.

Figure 52. Attempts to oxidise benzylic position in **472**.

Our next our attempt was driven towards oxidation of anionic intermediate **516**, which was envisioned to form upon treatment of pyridine **472** with a strong base such as LDA, Figure 53. Different electrophiles were used and the scope of them is presented in Table 7.

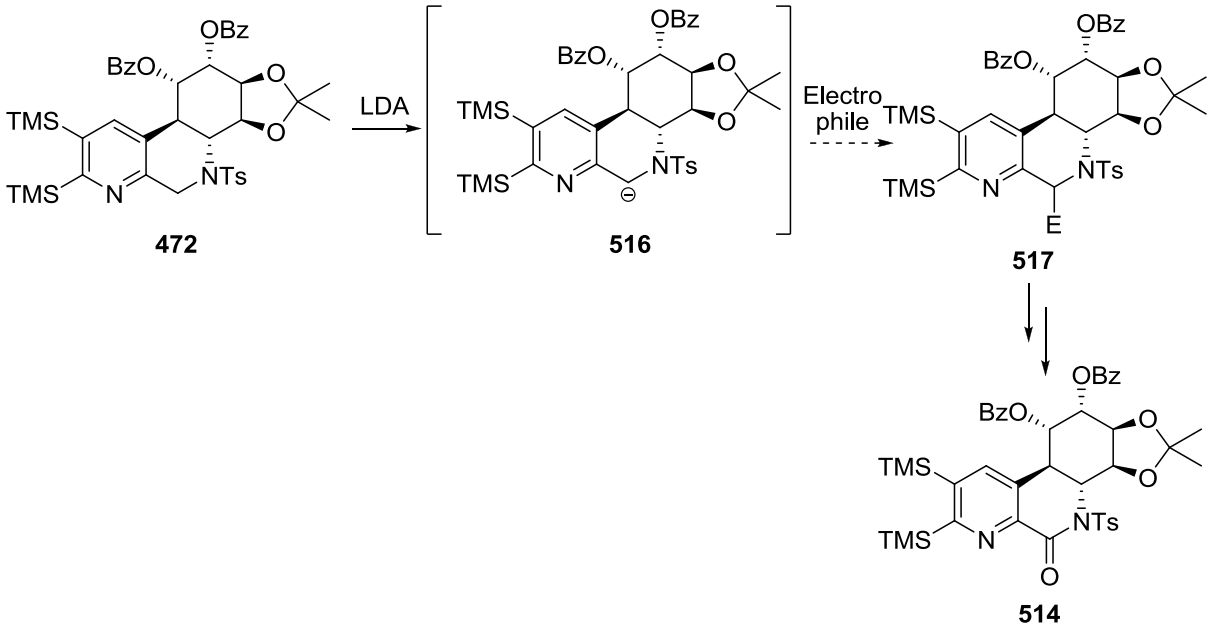
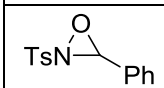


Figure 53. Deprotonation and functionalization of pyridine **472.**

The first attempt at oxidation was performed by molecular oxygen, however, no reaction was observed. The same results were observed with oxaziridine **517**. In order to observe some incorporation of deuterium, anion **516** was quenched with deuteromethanol, but the only isolated product was the debenzoylated pyridine **519**. Finally, in order to prove the formation of the transient anion **516**, the reaction was performed with TMSCl, the reagent known to trap anions *in situ*, but once again only starting material was recovered.

Table 7. Attempts to functionalize benzylic position in pyridine **472.**

Electrophile	Product
O_2	No reaction
 **517**	No reaction

CD_3OD	deprotection
TMSCl	No reaction

The last attempt to transform the benzylic position was envisioned *via* base-induced elimination of *p*-toluenesulfinate to form imine **518**, which was expected to serve as a precursor for the formation of amide group, Figure 54.

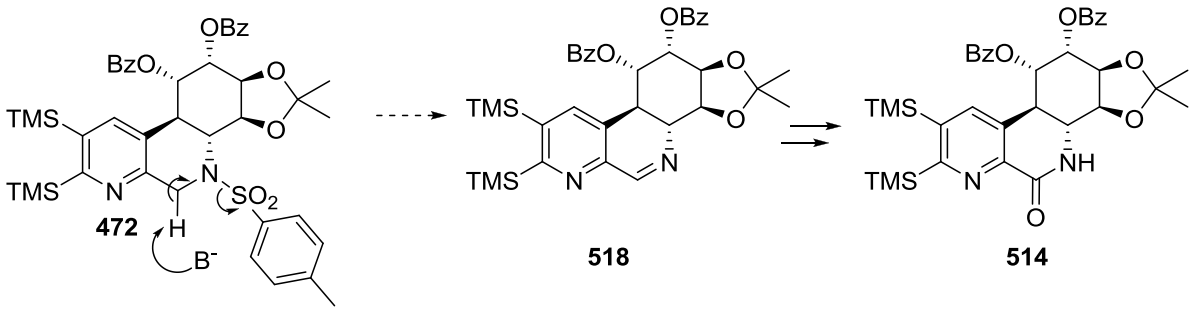
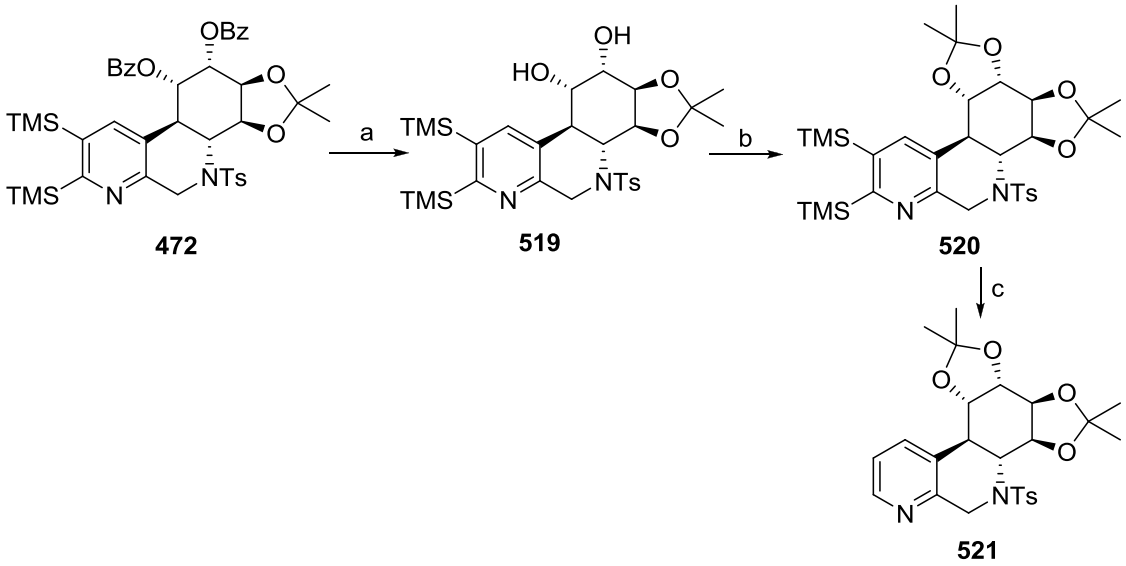


Figure 54. Base-induced formation of imine **518**.

The reaction was expected to proceed at high temperatures, so the base of choice had to be thermally stable and easy to remove upon purification. We decided to use KO*t*Bu, which can be easily sublimed and has the basicity sufficient to facilitate such rearrangement. Since the benzoates protecting groups in the pyridine **472** were incompatible with strong base such as KO*t*Bu, exchange of protection group was performed in a short sequence, Scheme 66. Deprotection by K_2CO_3 in methanol afforded diol **519**, followed by reprotection with the acetonide group provided *bis*-acetonide pyridine **520**, Scheme 66. Treatment of this compound with KO*t*Bu did not lead to formation of the imine but rather desilylation and elimination of the acetonide group was observed.



Reaction conditions: (a) K_2CO_3, CH_2Cl_2, MeOH, 62%; (b) 2,2-DMP, TsOH, 47%; (c) TBAF, THF, 70%.

Scheme 66. Synthesis of pyridine **521**.

In order to supress these unwanted reactions, desilylation of **520** was performed and isolated pyridine **521** was submitted to an NMR scale reaction in the presence of CD_3ONa. Formation of a new peak was observed in the alkene region, characteristic for the C-1 position, therefore we concluded that elimination of the acetonide occurred before elimination of *p*-toluene sulfinate on the NMR time scale. At this point it became clear that the cycloaddition route we pursued, requires major revisiting. Tricyclic alkene **522,** which was observed in minute quantities shares a similar structural pattern to narciclasine (**1**), so we decided to focus our attention on the synthesis of A-ring aza-analogues of narciclasine following a different route.

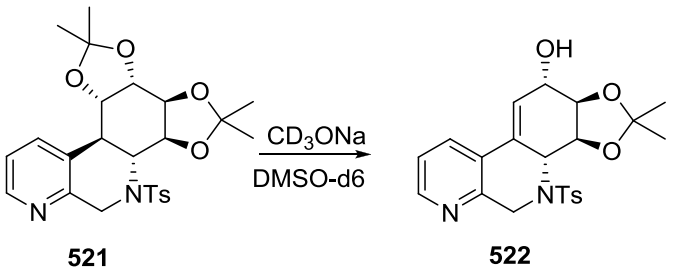


Figure 55. Base-induced elimination of acetonide in pyridine **521**.

3.4. Synthesis of pyridine analogues of narciclasine *via* intramolecular Heck approach

Our plan was to gain access to narciclasine aza-analogues in a convergent and short way, to produce several analogues having different position of nitrogen in the aromatic A-ring, and to study their biological activity. Our first goal was to design and perform the synthesis of 7-aza-8,9-dideoxynarciclasine **6** and its *N*-oxide **476**, which was of particular interest, since we thought, that N-O moiety can serve as a bioisostere for the hydroxyl in position C-7 of narciclasine and improve water solubility. After testing the validity of this approach, the synthesis of the more complex analogue 10-azanarciclasine (**7**) would be performed.

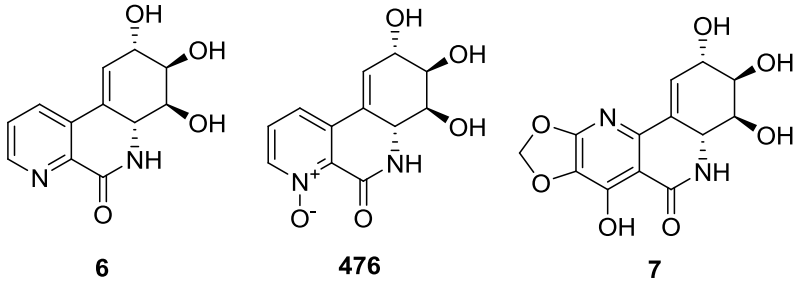


Figure 56. Aza-analogues of narciclasine.

3.4.1. Synthesis of 7-aza-8,9-dideoxynarciclasine.

In order to synthesize the desired analogues, we proposed to use an intramolecular Heck approach as one of the shortest and the most general approaches towards the narciclasine skeleton. Synthesis of both pyridine **5** and *N*-oxide **476** was envisioned *via* intramolecular coupling of tertiary amide **475**, which in turn was expected to be produced by coupling of 3-iodopicolinic acid **474** and protected conduramine **473**, Figure 57. Cyclohexadiene diol **4** was envisioned to be an ideal starting material for synthesis of conduramines such as **473**.

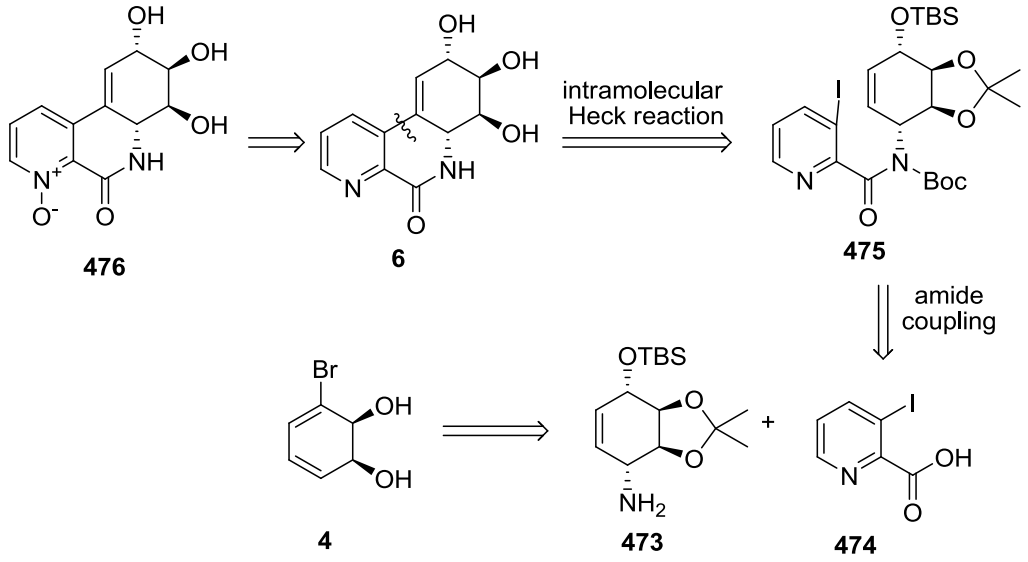
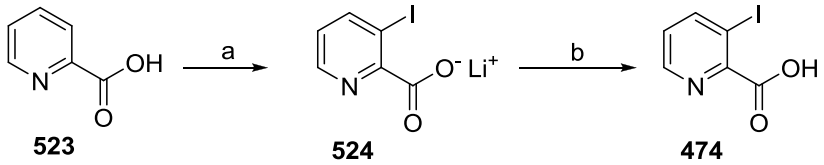


Figure 57. Retrosynthetic scheme towards 7-aza-8,9-dideoxynarciclasine **6**.

One of the most convenient ways to access halopyridinecarboxylic acid derivatives is to produce them *via* directed *ortho*-metallation (DoM).[225] In general, it requires the formation and hydrolysis of a strong directing group, such as a tertiary amide, which lengthens the synthesis. Nevertheless, it was observed by Queguiner[226] that in some cases unprotected pyridinecarboxylic acid can serve as a suitable substrate in DoM reaction with lithium tetramethylpiperidide (LTMP) as a base. This particular *ortho*-metallation

proceeds in good yield, but transformation of lithium 3-iodopicolinate (**524**) to the corresponding acid was reported to be surprisingly low-yielding, Scheme 67. This low yield can be attributed to the inherent instability of the free acid; in our hands samples of acid **474** produced by this procedure discoloured and started to decompose significantly upon standing at room temperature for a few days. Carson[227] modified this procedure in order to isolate pure lithium salt **524** in high yield. Since carboxylates can serve as a partners in amide coupling reactions[228] and salt **524** was significantly more stable than acid **474**, we decided to use it as a precursor for the coupling.

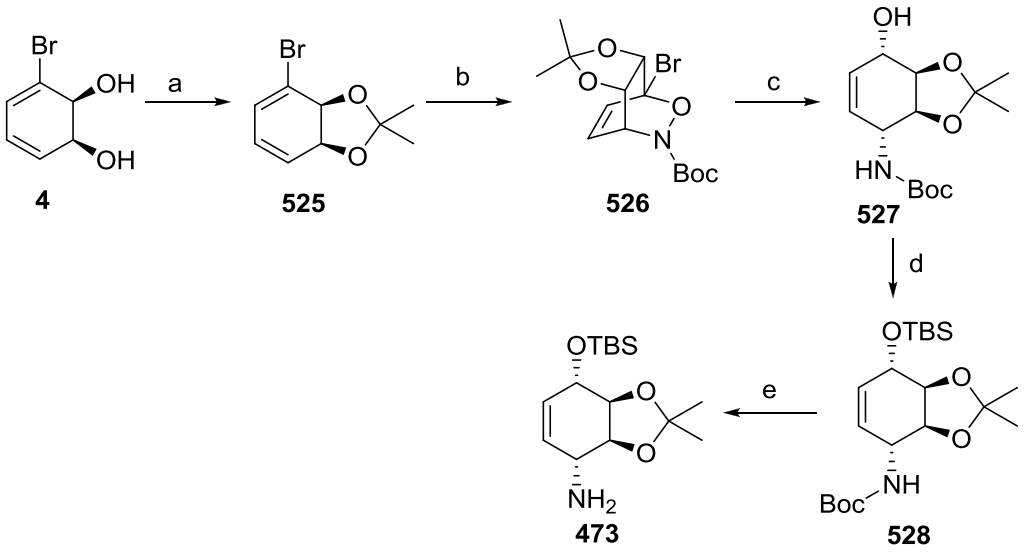


Reaction condtions: (a) (i) 2 eq. LTMP, 1 eq. *n*BuLi, -10 °C; (ii) I_2, 90%; (b) Amberlyst-15, MeOH, 35%.

Scheme 67. Directed ortho-metallation of picolinic acid.

Our next goal was to produce the amine partner for coupling. Hudlický and Olivo[137] developed an efficient route towards this type of compounds with effective utilisation of hetero-Diels-Alder reaction of dienes with nitroso compounds formed *in situ*. The synthesis started with the formation of the acetonide protected cyclohexadiene diol **525** which, without purification, was submitted to Diels-Alder reaction with the nitroso compound generated from *t*-butyloxycarbohydroxamic acid (BocNHOH)[229] to yield oxazine **526** with good yield, Scheme 68. Reduction of bicyclic oxazine **526** with sodium amalgam provided alcohol **527** with all stereocenters in a configuration identical to narciclasine. Further protection of the alcohol group was performed with TBSCl and imidazole in CH_2Cl_2 to provide completely protected conduramine **528**. Unfortunately

several conditions for the amide coupling including CDI, DCC/HOBT and $SOCl_2/Et_3N$ did not lead to coupling of amide **528** to 3-iodopicolinic acid **474**. Therefore selective deprotection of the *t*-butyloxycarbamate group was performed with trifluoacetic acid to yield the free amine **473.**

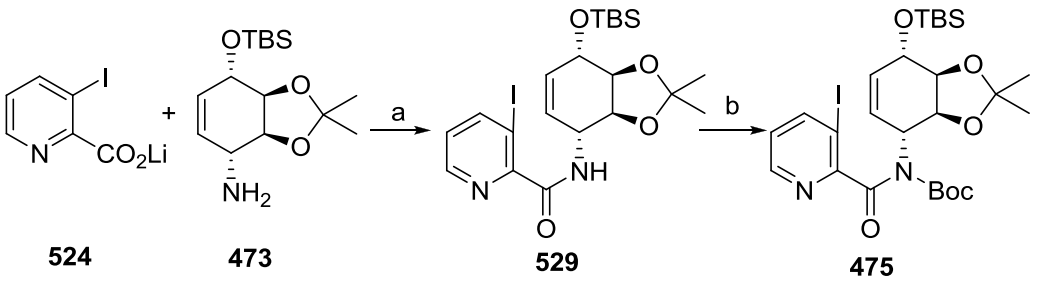


4 **525** **526** **527** **528** **473**

Reaction conditions: (a) 2,2-DMP, TsOH, acetone; (b) BocNHOH, $NaIO_4$, MeOH, EtOAc, H_2O, 74 % for two steps; (c) Al/Hg, THF, H_2O, 89%; (d) TBSCl, imidazole, CH_2Cl_2, 81%; (e) TFA, CH_2Cl_2, 86%.

Scheme 68. Synthesis of protected conduramine **473.**

Coupling of amine **473** with lithium carboxylate **524** was achieved under standard conditions with HBTU and diisopropylethylamine (DIPEA) in dry DMF, Scheme 69. The moderate yield of this reaction can be attributed to the steric hindrance of the iodo group and the generally lower reactivity of salts in coupling reactions. The next step consisted of transforming the secondary amide **529** to the protected amide **475** and this reaction proceeded in high yield.



Reaction conditions: (a) HBTU, DMF, DIPEA, 66%; (b) Boc_2O, DMAP, MeCN, 92%.

Scheme 69. Amide coupling of A- and C-ring precursors.

In all published intramolecular Heck approaches to the narciclasine or lycoricidine skeleton[32, 34, 36, 39, 40] the actual transformation is performed on a tertiary amide or imide and salts of silver or thallium are used as a base in order to facilitate the cationic pathway.[230] We decided to focus our attention on silver salts, since thallium salts are extremely toxic and less desirable for production of pharmaceuticals. Screening of different conditions for Heck coupling of amide **475** to naphthyridinone **530** was performed, Figure 58.

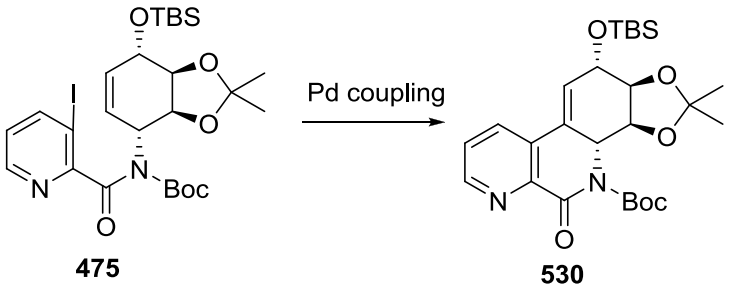


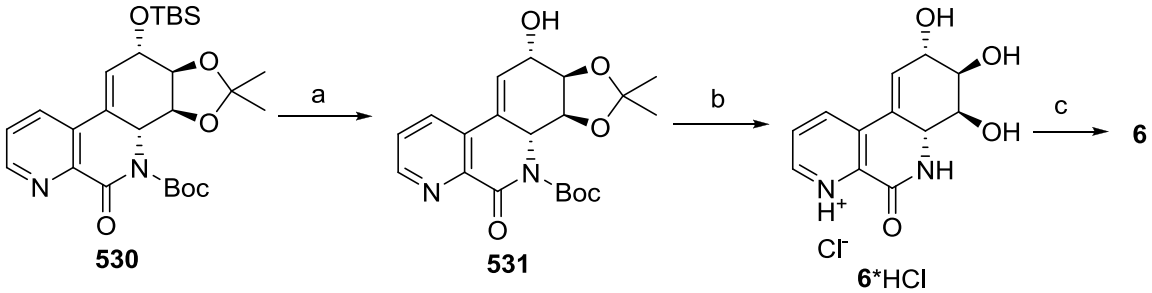
Figure 58. Heck intramolecular coupling.

Different phosphines, solvents, and bases were tried and the best yield was obtained in toluene in presence of dppe, Table 8. This reaction seems to be especially sensitive to the phosphine ligands, which is in accordance literature precendent that indicates bidentate phosphines tend to perform well in cationic pathway.[230]

Table 8. Screening of Heck conditions.

Conditions	Outcome
$Pd(dppf)_2Cl_2$, dppf, Ag_3PO_4 dioxane, 60°C, 24 h	No reaction
$Pd(OAc)_2$, dppe, Ag_3PO_4, Et_3N toluene, 80°C, 18 h	Traces of **530**
$Pd(OAc)_2$, PPh_3, Et_3N, $AgNO_3$, MeCN, 24 h, r.t to 80°C	No reaction
$Pd(OAc)_2$, dppe, $AgNO_3$, Cs_2CO_3, toluene, 24 h, 110°C	**530**, 35% (45 % brsm)
$Pd(OAc)_2$, BINAP, Ag_3PO_4, Et_3N toluene, 80°C, 18 h	No reaction

After developing the conditions for the key cyclization, our attention was driven towards deprotection of naphthyridinone **530** to desired compound 7-azanarcyclasine **6**, Scheme 70. Desilylation was performed under standard conditions with TBAF, followed by acidic deprotection of the acetonide and *t*-butyloxycarbamate group to attain the hydrochloride of the desired 7-aza-8,9-dideoxynarciclasine **6*HCl**. Upon reaction neutralization with concentrated ammonia and column chromatography was isolated free base **6**.



Reaction conditions: (a) TBAF, THF, 0°C, 77%, (b) HCl, MeOH, quant, (c) NH_3

Scheme 70. Deprotection of naphthyridinone **530**.

Study of the formation of *N*-oxide **476** from pyridine **6** was performed as shown Figure 59. Various conditions were tried[231] and the reaction turned out to be sensitive not just to the oxidant but also for the solvent in which reaction was performed, Table 9. The best conditions for transformation were found to be in use of recrystallized *m*CPBA as an oxidant in a mixture of dichloromethane and methanol. Conversion was slow and the reaction was low-yielding in general. Surprisingly enough, the solubility of pyridine **6** in water was observed to be much higher than corresponding *N*-oxide **476**. Biological studies were performed on 7-azanarciclasine **6**, the corresponding hydrochloride, and *N*-oxide **476**, and will be discussed in Section 3.5.

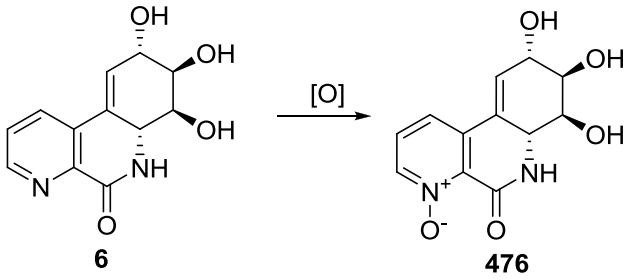


Figure 59. Selective oxidation of pyridine **6**.

Table 9. Conditions screening for formation of *N*-oxide **476**.

Conditions	Outcome
*m*CPBA, CH_2Cl_2	decomposition
*m*CPBA, CH_2Cl_2:MeOH=5:1	Traces of **476** and inseparable impurities
*m*CPBA, CH_2Cl_2:MeOH=10:1	Very slow conversion
*m*CPBA, CH_2Cl_2:MeOH=8:1	20% conversion to **476** in 48 h, 25% in a 120 h
TFA, H_2O_2 30% aq.	Decomposition

TFA, UHP (urea*H_2O_2), CH_2Cl_2	No reaction
UHP, CH_2Cl_2	No reaction
TFAA, UHP, CH_2Cl_2:MeOH	Traces of **476** and inseparable impurities

3.4.2. Synthesis of 10-azanarciclasine

After developing a short route to heterocyclic analogues of narciclasine we decided to test this approach on the synthesis of more complex target, namely 10-azanarciclasine (**7**). For the synthesis of **7** the convergence of our previous approach allowed to use of the same intramolecular Heck approach from amide **478**, and use of the same conduramine **473** as a precursor for the C-ring, Figure 60. The the only challenge was to develop a short and efficient synthesis of bromonicotinic acid **477**. We envisioned that this acid could be obtained by submission of bromopyridine **532** to the condition of the halogen dance or halogen scrambling with strong base, followed by quenching of the anionic intermediate with CO_2. Directed *ortho*-metallation of dioxolopyridine **533** followed by a borylation/oxidation/methylation sequence should provide access to bromopyridine **532**. In turn, the synthesis of pyridine **533** from furfural in two step was already published.[202]

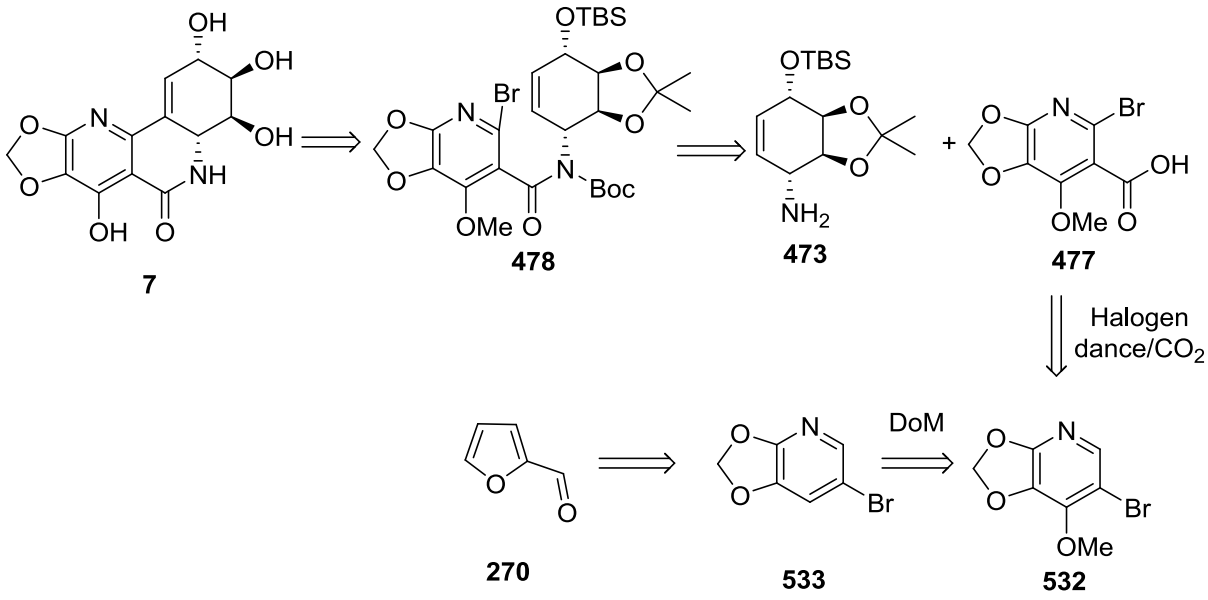
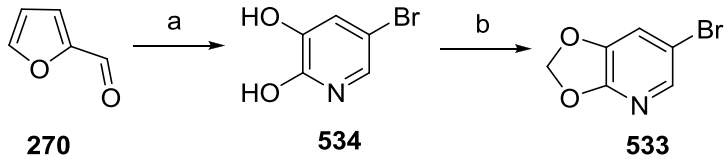


Figure 60. Retrosynthetic analysis for the synthesis of 10-azanarciclasine (**7**).

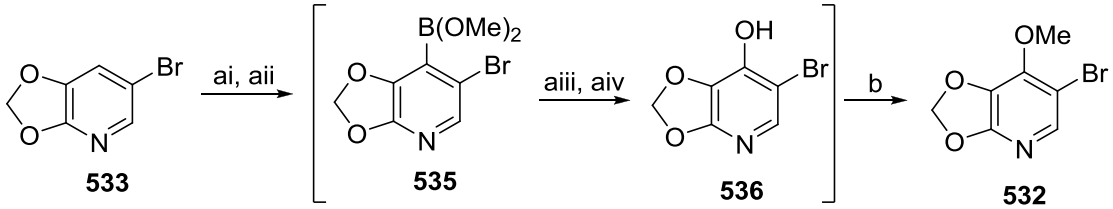
The key intermediate for this synthesis had to meet two requirements: (i) the presence of 2,3-dihydroxyl moiety in the pyridine ring, (ii) to be accessible in a short synthetic route from a common chemical. Synthesis of 6-bromo[1,3]dioxolo[4,5-*b*]pyridine (**533**) was published by Dallacker[202] complied with both of these conditions. In our hands, the intermediate 2,3-dihydroxy-5-bromopyridine (**534**) was obtained by treatment of furfural (**270**) with bromine and sulfamic acid under acidic conditions in a 65% yield, slightly higher than in the original publication (48%), and after treatment with CH_2Br_2 a methylenedioxy bridge was installed in low yield. This yield, while low, is in accordance with limited examples of similar reactions in the literature.[232]



Reaction conditions: (a) (i) 1 eq. Br_2, H_2O, 0°C; (ii) HCl, H_2O, 0°C; (iii) 1 eq. Br_2, 0°C; (iv) H_2NSO_3H, 50°C, 65%, (b) CH_2Br_2, K_2CO_3, CuO, DMF, 18%.

Scheme 71. Synthesis of intermediate **533**.

Our next goal was to synthesise 6-bromo-7-methoxy[1,3]dioxolo[4,5-*b*]pyridine (**532**) from pyridine **533**, Scheme 72. The most logical way was to perform the sequence DoM with LTMP, followed by borylation with $B(OMe)_3$ and *in situ* oxidation with the urea-hydrogen peroxide complex. It is worth mentioning, that this oxidation is sensitive to moisture and in the presence of usual oxidants for such transformation, such as 30% aq. H_2O_2, led to complete protiodeborylation and only starting material **533** was isolated. Transient 4-hydroxypyridine **536** was not isolated but instead submitted directly to the methylation step without purification. In order to perform selective *O*-alkylation, diazomethane was used, since reaction did not proceed selectively with dimethylsuflate, methyl iodide, and Meerwein salt. The desired methoxypyridine **532** was isolated in a moderate yield and despite the persisting presence of unidentified impurity was successfully employed in the next steps.



Reaction conditions: (a) LTMP,THF, -78°C; (ii) $B(OMe)_3$; (iii) AcOH; (iv) UHP; (b) CH_2N_2, THF, 36%.

Scheme 72. Synthesis of intermediate **532**.

Having secured access to the key intermediate **532**, the next goal was to the study halogen dance reaction of this bromopyridine upon treatment with LTMP, Figure 61. In order to provide evidence of formation of aryllithium intermediate **537** it was quenched with two electrophiles, methanol and DMF to provide isomeric halopyridines **538** and aldehyde **539** respectively. Spectroscopic NMR characteristics of **538** and **539**, were sufficient to provide the definitive proof of structure of regioselective formation of aldehyde **539**.

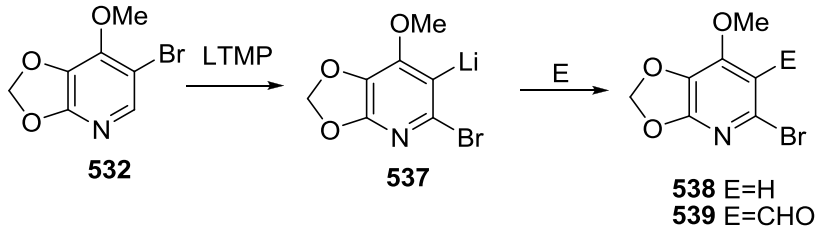


Figure 61. Halogen dance reaction.

The halogen dance reaction on similar systems has some precedence in the literature, especially in the field of total synthesis of polyoxygenated pyridine natural products (see section 2.3.4). However, it is worth mentioning that all published procedures report the direct addition of the substrate to the organometallic reagent (LTMP). In our hands these conditions did not provide the desired product, but at low temperature (−90 – −80°C) led to simple deprotonation of α-position, and at higher temperatures (−75 – −70°C) decomposition was observed. Reverse slow addition of LTMP to the substrate **532** led to conversion to the intermediate aryllithium intermediate **537**, which is in accordance with the current hypotheses of the mechanism of this reaction.[233] In order to prove this observation we decided to match products of electrophile quench, obtained by halogen dance reaction of **532**, with the product of the direct addition of base to 2-bromopyridine

538. To our satisfaction both of these reaction proceeded well and yielded upon quench with CO_2 the same lithium carboxylate **540**, Figure 62.

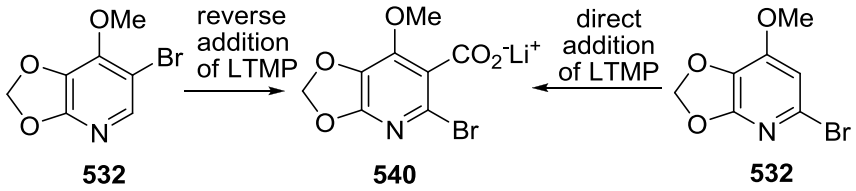
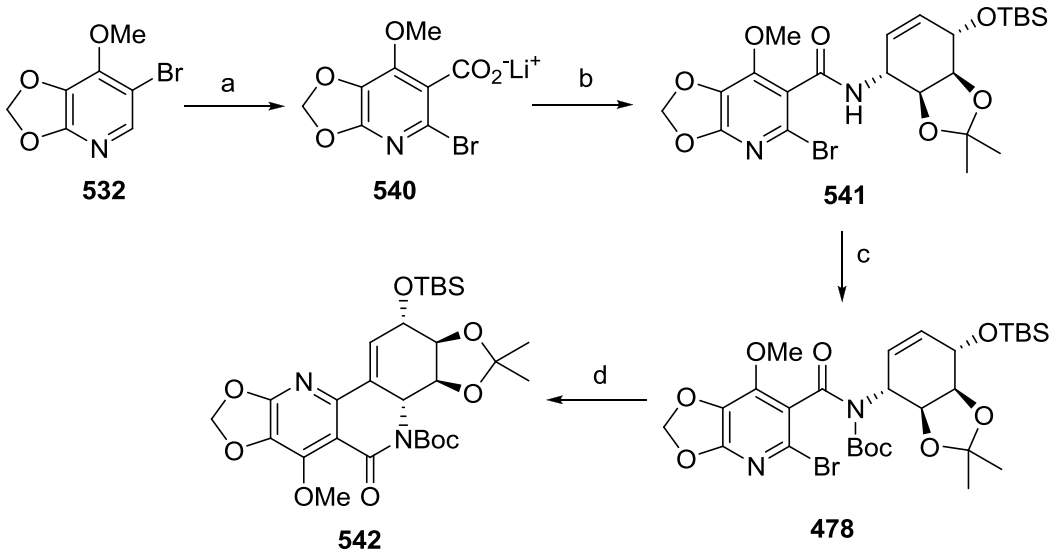


Figure 62. Formation of carboxylate **540** upon different conditions.

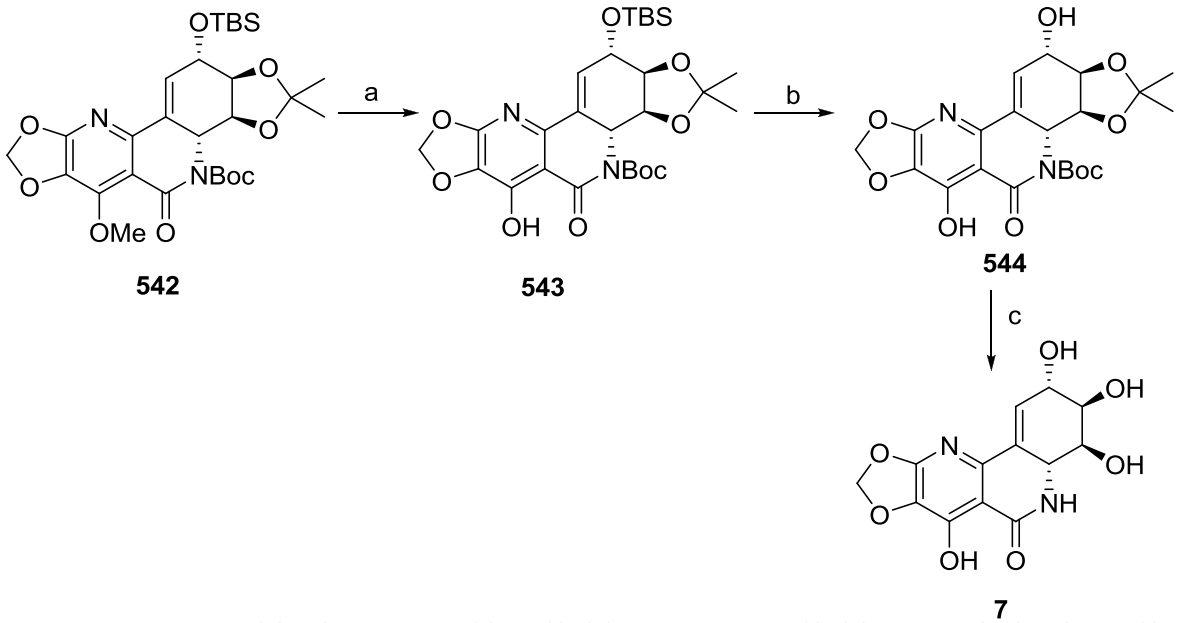
In order to attain the key intermediate for the Heck reaction, namely amide **478**, we decided to proceed to the coupling of amine **473** without isolation of the carboxylic acid salt **540**, Scheme 73. Bromopyridine **532** was used as the starting material, since it provided shorter access to the desired compound. Compound **532** was submitted to the halogen dance reaction in the presence of LTMP, followed by electrophilic quench with solid CO_2. The reaction proceeded well, and was immediately followed by transformation to the amide **478** under conditions of HBTU coupling. For the intramolecular Heck cyclization, conditions similar to those described before were used. The only difference was that the reaction was performed at lower temperature and therefore a longer reaction time was required. Due to this modification the fully protected 10-azanarciclasine (**542**) was isolated in higher yield than the corresponding 7-azacompound.



Reaction conditions: (a) (i) LTMP, THF, -80°C; (ii) CO_2; (b) **473**, HBTU, DIPEA, MeCN, 30% for 2 steps; (c) Boc_2O, DMAP, MeCN, 80%; (d) $Pd(OAc)_2$, dppe, Ag_3PO_4, Cs_2CO_3, PhMe, 90°C, 58%.

Scheme 73. Synthesis of the fully functionalized skeleton of 10-azanarciclasine.

Deprotection of naphthyridinone **542** was performed under the same conditons as C-1 analogues, see Scheme 62. Demethylation of **542** was achieved by treatment with fused lithium chloride in DMF at 100 °C, Scheme 74. Product **543** was isolated and submitted to desilylation conditions in the presence of TBAF, which led to a clean conversion to alcohol **544,** which in turn was submitted to acid-catalyzed deprotection conditions in order to remove the acetonide and the *t*-butyloxycarbonyl group. Different conditions for deprotection were tried: strong acid such as HCl in methanol led to a significant decomposition of the starting material, and weaker acids such as formic or trifluroacetic did not lead to deprotection. The best results were obtained with excess of wet (H_2O 5% v/v) trifluoroacetic acid, yielding **7** in good yield.The synthesis of this analogue was achieved in 11 steps of longest linear sequence (9 one-pot reactions).



Reaction conditions: (a) LiCl, DMF, 100°C, 56%; (b) TBAF, THF, 90%; (c) TFA, H_2O, CH_2Cl_2, 77%.

Scheme 74. Deprotection of 10-azanarciclasine.

3.5. Biological activities of unnatural analogues

The strategies described above provided access to the three new analogues of pancratistatin with substituent on C-1 and three new analogues of narciclasine with a nitorgen placement in the A-ring. These new Amaryllidaceae constituents were tested *in vitro* against a panel of cancer cell lines through collaboration with the groups of Dr. Kornienko and Dr. Rodelj from New Mexico Institute of Mining and Technology, NM, Dr. Pandey from University of Windsor, ON, and the group in the Center for Research and Drug Develoment, BC.

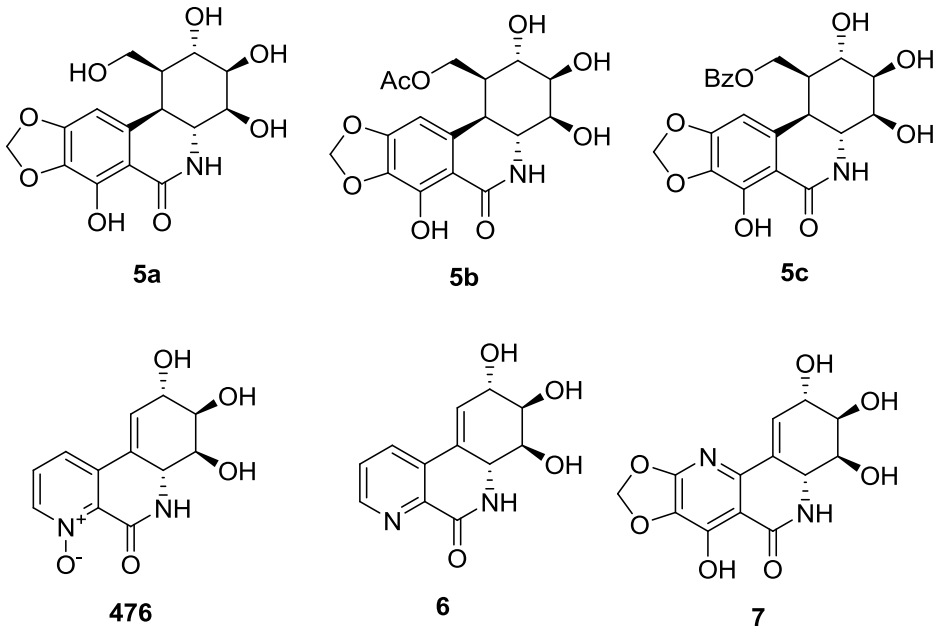


Figure 63. Unnatural analogues of pancratistatin and narciclasine.

The C-1 analogues of pancratistatin were expected to have high activity since our group has previously reported[79] that C-1 analogues of 7-deoxypancratistin (**11**), namely the alcohol **192a** and acetate **192b**, displayed antiproliferative activity equal to that of the parent compound **11**. Since the parent compound **2** of our analogues displays activity 10-100 times higher than **11**, we had reasonable expectations that our C-1 compounds and especially benzoate **5c** would display activity similar to the benzoate ester **294** because of their very similar structures. The results of the antiproliferative activity are presented in Table 10.

Table 10. Activity of C-1 analogues with narciclasine as standard (IC_{50}, EC_{50}, μM)

Cell line (type of cancer)	Compound **5a**	Compound **5b**	Compound **5c**	Narciclasine (**1**)
BxPC-3	0.22 ± 0.01	0.07 ± 0.01	**0.01 ± 0.00**	0.05 ± 0.01

(Pancreatic)				
DU-145 (Prostate)	0.09 ± 0.01	0.06 ± 0.01	**0.01 ± 0.00**	0.03 ± 0.01
NCI-H460 (Lung)	0.09 ± 0.01	0.07 ± 0.01	**0.03 ± 0.01**	0.05 ± 0.03
MCF-7 (Breast)	0.24 ± 0.10	0.52 ± 0.47	**0.08 ± 0.01**	0.06 ± 0.03
HCC1954 (Breast)	0.163	0.086	**0.011**	
Jurkat (Leukemia)	0.266	0.211	**0.007**	

As it can be seen from the table, C-1 benzoate homologue **5c** displays particularly strong antiproliferative activity, exceeding even the natural congener narciclasine (**1**), which is amongst the most active natural products in this family. It further underlines the previously observed[48, 103] increased beneficial effect of large lipophilic groups, especially benzoates, on the position C-1, on activity. At the same time, despite the pronounced activity, our pancratistatin homologue compound **5c** did not quite reach activity of C-1 benzoate pancratistatin ester (**294**). These results show that there are some limitations on the length and size of the lipophilic group on the position C-1.

Parallel studies have also been performed on different cancer lines, namely colorectal cancer HCT 116 and osteosarcoma Saos-2 cell lines and are presented in the following Figure 64. The difference between 7-deoxypancratistatin C-1 homologue alcohol **192a** and acetate **192b**, and their respective pancratistatin counterparts **5a** and **5b** can be clearly seen, with the latter being significantly more active. This fact once again shows the

importance of the presence of the 7-hydroxyl group in order to display high antiproliferative potency.

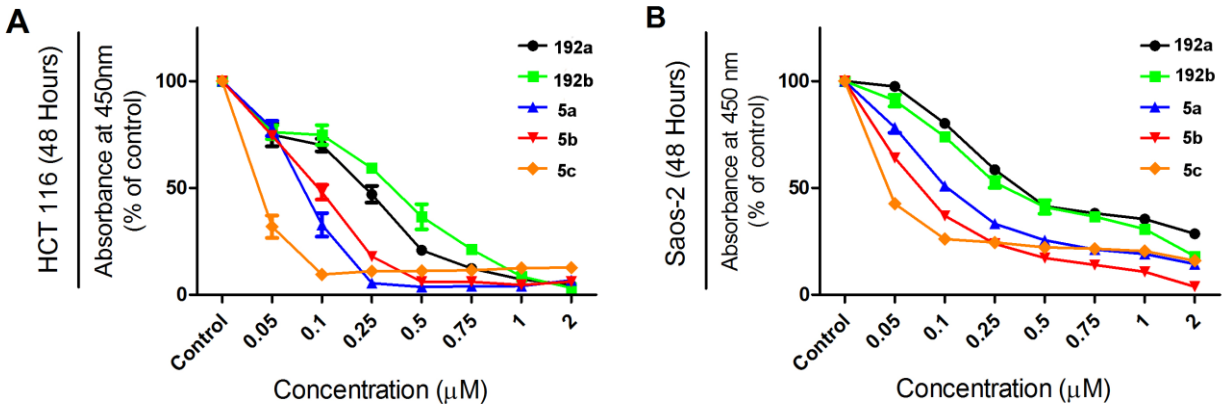


Figure 64. Cytotoxicity of C-1 analogues in dose dependant manner.

Next goal was to test the heterocyclic analogues of narciclasine we prepared. Three compounds were tested against a panel of cell lines and the results are presented in Table 11. It can be seen from the table, that 7-aza compounds show no activity (IC_{50}>100 μM) against two different cancer cell lines. This result might be explained by the belief that requirement of the C-8 and C-9 alkoxy groups that may be necessary to retain activity. A second reason might be that these compounds have very different physicochemical properties from parent narciclasine, such as high aqueous solubility and low lipophilicity.

Table 11. Activity of 7-aza analogues of narciclasine (IC_{50}, μM, extrapolated)

Cell line (type of cancer)	Compound **6**	Compound **6*HCl**	Compound **476**
HeLa (Cervical)	452	152	300

MCF-7 (Breast)	331	196	183

Testing performed by CDRD on a two different cancer cell of all four azaanalogues confirmed our previous observations, Table 12. 7-aza compounds displayed no cytotoxicity at low micromolar concentrations and, in turn, 10-azanarciclasine showed only reduced activity in comparison with the natural congener narciclasine. These results further support our conclusion about requirements of fully oxygenated A-ring for retaining anticancer activity and that nitrogen in A-ring plays deleterious role in pharmacophore.

Table 12. Activity of 7-aza and 10-aza analogues of narciclasine (IC_{50}, μM)

Cell line (type of cancer)	Compound **6**	Compound **6*HCl**	Compound **476**	Compound **7**
Jurkat (Leukemia)	Inactive[f]	Inactive	Inactive	0.5
HCC1954 (Breast)	Inactive	Inactive	Inactive	0.5

[f] Showed no cytotoxicity at 2.7 μM concentration.

4. Conclusions and Future work

The Amaryllidaceae constituents narciclasine (**1**) and pancratistatin (**2**) are isocarbostyril natural products which possess strong anticancer activity. In the course of the present study three different approaches towards analogues of these two natural products based on chemoenzymatic methods were studied. Two of these of approaches resulted in efficient synthetic routes and led to the synthesis of several structurally diverse C- and A-ring analogues of pancratistatin and narciclasine repsectively.

The synthesis of six new analogues was accomplished and two of these analogues, namely C-1 acetate **5b** and C-1 benzoate **5c** have shown significant activity against a panel of cancer cell lines. The two truncated heterocyclic analogues, 7-aza-8,9-dideoxynarciclasine **6** and its *N*-oxide **476** displayed no activity, and one heterocyclic analogue **7** showed only low activity.

Derivatization at the C-1 position was shown to be beneficial in increasing the potency of natural compounds. Also it was shown that truncated 7-aza analogues do not possess any antiproliferative activity and therefore the alkoxy groups, are likely a requirement for the minimal pharmacophore. Bioisosteric replacement with nitrogen apparently plays a deleterious role in the anticancer activity of compound, but in the same time azanarciclasine analogues exhibit higher aqueous solubility than natural compounds **1** and **2**.

Future studies are necessary in order to further probe the minimal pharmacophore requirements of the aromatic A-ring and to refine the synthesis of the C-ring analogues in

terms of step count and overall efficiency. New route to the C-1 analogues will be required, in order to refine existing synthesis, to avoid toxic reagents, and shorten step count to meet strict demands of pharmaceutical industry. Also, the C-1 pancratistatin analogues could be obtained through efficient borylation and hydroboration transformation of narciclasine-type compounds. The reason for that, is that narciclasine compounds can be synthesised in much more efficient ways and can also be isolated from natural sources.

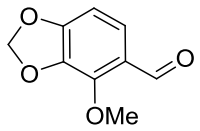
5. Experimental section

5.1. General Experimental section.

Reactions were carried out under inert atmosphere in oven-dried or flame-dried glassware unless stated otherwise. LiCl was fused under vacuum immediately before use. Solvents were distilled prior to use: CH_2Cl_2, DMF, iPr_2NEt, and pyridine from CaH_2, MeOH from magnesium methoxide, THF and DME from Na/benzophenone, toluene from Na, quinoline from Zn. Qualitative TLC was done with precoated silica gel aluminum sheets (EMD silica gel 60 F_{254}), detection by UV or by spraying with "CAM" solution (5 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 1 g of $Ce(SO_4)_2$, 100 mL of 10% H_2SO_4) or 0.5% aqueous $KMnO_4$ solution followed by heating. Melting points are uncorrected. Flash chromatography was performed using silica gel SiliaFlash P60 from Silicycle (40–66 μm). Optical rotation was measured in a 1-dm cell at 20-25 ˚C and 589 nm, concentration *c* in g/100 mL, specific rotation measurements are given in deg cm^3g^{-1} dm^{-1} and were recorded on a Perkin-Elmer 341 polarimeter, IR spectra were recorded on Perkin-Elmer FT-IR 1600 Series Spectrum One instrument in KBr pellets or as thin films. ^1H NMR and ^{13}C NMR spectra were recorded at 300 or 600 MHz and 75 or 150 MHz, respectively, and were calibrated on the solvent residual peak or TMS signal ($CDCl_3$ - 7.28 ppm; DMSO - d6 - 2.51), the chemical shifts are reported in ppm. Data are reported as (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad); coupling constants(s) in Hz, integration.

5.2. Detailed experimental section.

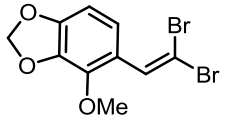
4-methoxy-1,3-benzodioxole-5-carbaldehyde (486)



Dimethyl sulfate (22.7 mL, 240 mmol) was added to a mixture of K_2CO_3 (55.2 g, 400 mmol) and phenol **484**[213] (33.2 g, 200 mmol) in acetone (260 mL). The reaction mixture was stirred under reflux until consumption of starting material (TLC, approximately 4 h). Then reaction mixture was cooled and inorganic salts were removed by filtration and rinsed with acetone (2×100 mL). The solution was evaporated, redissolved in CH_2Cl_2, washed sequentially with 10 % solution of NaOH, water, and saturated solution of NaCl, then organic solution dried over anhydrous Na_2SO_4 and evaporated to obtain compound **486** (30.2 g, 84%) as pale brown crystals, which was used without further purification.

R_f 0.65 (Hexanes/EtOAc 9:1); mp 102-104 °C (EtOH); [Lit. value[234] 103-105 °C (EtOH)]; ^{1}H NMR ($CDCl_3$, 300MHz): δ 10.24 (s, 1H); 7.49 (d, J = 8.3 Hz, 1H), 6.62 (d, J = 8.3 Hz, 1H), 6.05 (s, 2H), 4.14 (s, 3H).

5-(2,2-dibromovinyl)-4-methoxy-1,3-benzodioxole (487)

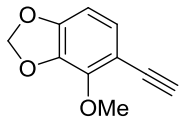


Triphenylphosphine (64.0 g, 244 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a stirring solution of CBr_4 (40.5 g, 122 mmol) in CH_2Cl_2 (150 mL) at 0 °C (ice bath). After 15 min of stirring, a solution of aldehyde **486** (11.0 g, 61.0 mmol) in CH_2Cl_2 (50 mL) was added dropwise. Upon completion the reaction was reduced in volume to 100 mL

and slowly poured into vigorously stirred hexanes (1400 mL). Then mixture was filtered through short plug of silica, washed with a mixture of hexanes:EtOAc (10:1, 200 mL) and evaporated. Subjection of this material to flash column chromatography (Eluent hexanes/EtOAc 9:1) and concentration of the relevant fractions gave **487** (16.11 g, 78.6%) as a white solid.

R_f 0.9 (Hexanes/EtOAc 9:1); mp 38 - 40 °C (pentane); IR (KBr, cm^{-1}) ν 3448, 2981, 2948, 2934, 2900, 2876, 2838, 2770, 1625, 1605, 1471, 1427, 1384, 1350, 1265, 1213, 1126, 1072, 1045, 979, 960, 939, 929, 848, 829, 788, 767, 729, 644; ^{1}H NMR ($CDCl_3$, 300MHz) δ 7.49 (s, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 6.57 (d, *J* = 8.3 Hz, 1H), 5.96 (s, 2H), 4.02 (s, 3H); ^{13}C NMR (CDCl3, 75MHz) δ 149.7, 141.1, 136.1, 132.3, 122.5, 121.4, 102.3, 101.2, 89.0, 59.9; MS (+EI) *m/z* (%): 338 (49) [^{81}Br+^{81}Br, M]$^+$, 336 (100) [^{81}Br+^{79}Br, M]$^+$, 334 (51) [^{79}Br+^{79}Br, M$^+$], 242 (55), 240 (57), 176 (53), 175 (42), 131 (29); HRMS (+EI) calcd for $C_{10}H_8Br_2O_3$: 333.8820; found 333.8845; Anal. Calcd for $C_{10}H_8Br_2O_3$: C, 35.75; H, 2.40. Found C, 35.99; H, 2.41.

5-ethynyl-4-methoxy-1,3-benzodioxole (482)

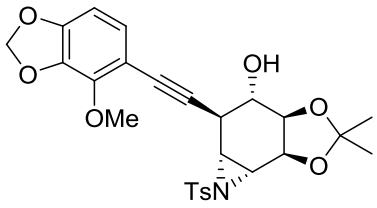


To a solution of **487** (19.38 g, 57.68 mmol) in THF (350 mL) was added solution of *n*BuLi (52.9 mL, 2.5 M, 130 mmol) at -78 °C. After 20 min of stirring at −78 °C, the reaction mixture was warmed to room temperature over a period of 2 h. A saturated solution of NH_4Cl (40 mL) was poured into reaction mixture, which was later extracted

by CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated. The crude compound was purified by flash chromatography (hexanes:EtOAc 2:1) and **482** was obtained as white crystals (8.5 g, 81.7%).

R_f 0.9 (Hex:EtOAc 2:1); mp 77-78 °C (pentane); IR (KBr, cm^{-1}) ν 3278, 3254, 3000, 2945, 2901, 2846, 2794, 1620, 1600, 1469, 1433, 1336, 1267, 1229, 1077, 1043, 979, 950, 930, 797; ^1H NMR ($CDCl_3$, 300MHz) δ 7.01 (d, J = 7.9 Hz, 1H), 6.50 (d, J = 8.29 Hz, 1H), 5.98 (s, 2H), 4.11 (s, 3H), 3.20 (s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 150.1, 144.8, 136.2, 128.0, 108.1, 102.9, 101.3, 79.9, 79.4, 60.0; MS (+EI) *m/z* (%): 176 $[M]^+$ (100), 175 (29), 131 (16), 53 (18); HRMS (+EI) calcd for $C_{10}H_8O_3$: 176.0473; found, 176.0475; Anal. calcd for $C_{10}H_8O_3$: C, 68.18; H, 4.58. Found C, 68.27; H, 4.55.

(3a*S*,4*R*,5*R*,6*R*,7*S*,7a*R*)-6-[(4-methoxy-1,3-benzodioxol-5-yl)ethynyl]-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxol-7-ol (490)

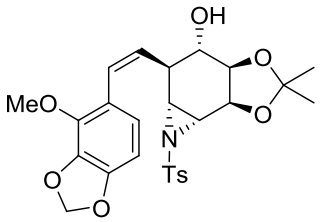


To a solution of alkyne **482** (1.580 g, 8.95 mmol) in toluene (30 mL) at -50 °C *n*-BuLi (3.80 mL, 2.35 M, 8.95 mmol) was added dropwise. After 15 min of stirring Me_2AlCl (9.0 mL, 1.0 M, 8.95 mmol) was added dropwise. The reaction mixture was warmed to 0 °C within 1 h and stirred for an additional 40 min at 0 °C. The reaction mixture allowed to warm room to temperature and stirred for 40 min. The reaction mixture was cooled to -30 °C and a solution of epoxide **184 (**1.510 g, 4.47 mmol**)** in toluene (20 mL) was added dropwise. The reaction mixture was stirred for 1 h and was allowed to warm to room

temperature overnight. The reaction mixture was cooled to 0 °C by ice bath and quenched with 1 N HCl (1 mL), followed by ice-cold water (1 mL) and 1 N HCl (2 mL). Reaction mixture was filtered through a plug of Celite® and extracted with EtOAc (3 × 20 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (gradient hexanes/EtOAc 5:1->3:1) to give product as colorless oil (1.02g, 44%).

R_f 0.3 (Hex/EtOAc 2:1);$[\alpha]^{24}_D$ + 78.4 (*c* = 1.0, $CHCl_3$); IR (film, cm^{-1}) ν 3482, 3093, 2986, 2935, 2900, 1620, 1599, 1470, 1434, 1404, 1383, 1332, 1307, 1260, 1225, 1186, 1183, 1071, 985; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.83 (d, *J* = 8.3 Hz, 2H), 7.39 (d, *J* = 8.3 Hz, 2H), 6.88 (d, *J* = 8.1 Hz, 1H), 6.47 (d, *J* = 8.3 Hz, 1H), 5.96 (s, 2H), 4.48 (d, *J* = 6.2 Hz, 1H), 4.19 (t, *J* = 5.7 Hz, 1H), 4.06 (s, 3H), 3.98-3.95 (m, 1H), 3.44-3.41 (m, 1H), 3.26 (d, *J* = 6.8 Hz, 1H), 3.24 (dd, *J* = 5.0, 2.1 Hz, 1H), 3.08 (d, *J* = 8.7 Hz, 1H), 2.48 (s, 3H), 1.51 (s, 3H), 1.34 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 149.7, 145.4, 144.4, 136.3, 134.1, 130.1, 127.9, 127.2, 110.1, 108.8, 102.8, 101.3, 87.6, 80.5, 75.5, 70.3, 69.0, 60.0, 42.3, 40.2, 31.9, 27.3, 25.1, 21.7; MS (+FAB) *m/z* (%): 514 (24) $[M+H]^+$, 513 (13) $[M]^+$, 258 (12), 238 (11), 230 (14), 179 (26), 155 (28), 149 (23), 43 (100); HRMS (+FAB) calcd for $C_{26}H_{28}NO_8S^+$ $[M+1]^+$: 514.1436; found, 514.1502.

(3a*S*,4*R*,5*R*,6*R*,7*S*,7a*R*)-6-[(*Z*)-2-(4-methoxy-1,3-benzodioxol-5-yl)vinyl]-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxol-7-ol (492)

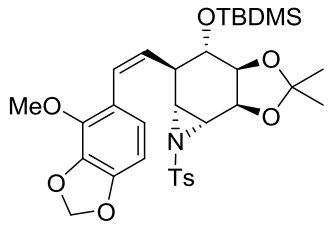


To a solution of compound **490** (427 mg, 0.83 mmol) in MeOH (20 mL) was added quinoline (11 mg, 0.09 mmol). The solution was charged with Lindlar catalyst (5%, 100 mg) and allowed to stir under H_2 (1 atm) for 45 min. After consumption of starting material (NMR), the reaction mixture was filtered through a pad of Celite®, washed with CH_3OH (3 × 30 mL), evaporated, and used without further purification. Analytical sample was purified by column chromatography (hexanes/EtOAc 3:1).

R_f 0.4 (hexanes/EtOAc 2:1); $[\alpha]^{20}_D$ + 2.7 (*c* = 1.78, $CHCl_3$); IR (film, cm^{-1}) ν 3482, 2987, 2934, 2900, 1621, 1598, 1470, 1434, 1404, 1382, 1332, 1260, 1218, 1162, 1070, 1043; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, *J* = 8.3 Hz, 2H), 7.32 (d, *J* = 8.3 Hz, 2H), 6.60 (d, *J* = 11.4 Hz, 1H), 6.55 (d, *J* = 8.1 Hz, 1H), 6.46 (d, *J* = 8.0 Hz, 1H), 5.98 (d, *J* = 1.2 Hz, 1H), 5.96 (d, *J* = 1.2 Hz, 1H), 5.73 (t, *J* = 11.2 Hz, 1H), 4.43 (d, *J* = 6.2 Hz, 1H), 4.16-4.13 (m, 1H), 3.96 (s, 3H), 3.72-3.68 (m, 1H), 3.18 (d, *J* = 6.4 Hz, 1H), 3.15-3.10 (m, 1H), 3.08 (d, *J* = 6.4 Hz, 1H), 2.84 (d, *J* = 9.2 Hz, 2H), 2.43 (s, 3H), 1.49 (s, 3H), 1.30 (s, 3H). ^{13}C NMR ($CDCl_3$, 75MHz) δ 148.9, 145.2, 141.3, 136.6, 134.3, 130.0, 128.1, 127.9, 127.8, 122.5, 122.2, 109.7, 102.6, 101.0, 75.8, 70.1, 69.3, 59.7, 43.1, 40.5, 37.8, 27.1, 24.7, 21.7.MS (+FAB) *m/z* (%) $[M]^+$: 517 (16), 516 (42), 515 (29), 514 (15), 386 (25), 285 (14), 284 (15), 269 (15), 203 (30), 165 (61), 91 (100); HRMS (+FAB) calcd for

$C_{26}H_{30}NO_8S$ $[M+1]^+$: 516.1692; found, 516.1666. Anal. calcd for $C_{26}H_{29}NO_8S$: C, 60.57; H, 5.67. Found C, 60.33; H, 5.55.

(3aS,4R,5R,6R,7S,7aR)-7-{tert-butyl[dimethylsylil]oxy}-6-[(Z)-2-(4-methoxy-1,3-benzodioxol-5-yl)vinyl]-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxol (481)

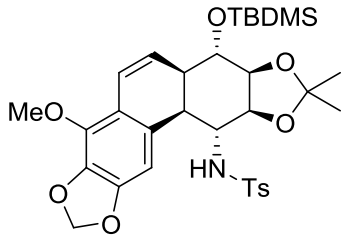


To a solution of alcohol **492** (1.07 g, 2.07 mmol) in 30 mL of CH_2Cl_2 was added Et_3N (0.58 mL, 4.15 mmol) at 0 °C and *t*-butyldimethylsilyl triflate (0.53 mL, 2.29 mmol) was added dropwise. After complete consumption of starting material (TLC) reaction mixture was quenched by water (10 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with 10 % citric acid (5 mL), brine (5 mL), dried over Na_2SO_4, and concentrated to afford **481** as pale yellow oil (1.26 g, 97%). The Compound was used without further purification. Analytical sample was purified by silica gel chromatography (hexanes/EtOAc 4:1).

R_f 0.85 (hexanes:EtOAc 2:1); $[\alpha]^{24}_D$ − 18.5 (*c* = 2.0, $CHCl_3$); IR (KBr, cm^{-1}) ν 3446, 2986, 2954, 2931, 2887, 2856, 1622, 1600, 1470, 1435, 1382, 1332, 1257, 1218, 1163, 1071, 1043; ^1H NMR ($CDCl_3$, 300MHz): δ 7.78 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 6.67 (d, *J* = 8.0 Hz, 1H), 6.60 (d, *J* = 11.5 Hz, 1H), 6.39 (d, *J* = 8.0 Hz, 1H), 5.96-5.93 (m, 2H), 5.65 (t, *J* = 11.5 Hz, 1H), 4.39 (d, *J* = 5.9 Hz, 1H), 3.98 (s, 3H), 3.89 (t, *J* = 6.03 Hz, 1H), 3.67 (t, *J* = 6.3 Hz, 1H), 3.12 (d, *J* = 6.6 Hz, 1H), 2.98-2.91 (m, 2H), 2.45

(s, 3H), 1.52 (s, 3H), 1.34 (s, 3H), 0.78 (s, 9H), 0.00(s, 3H), −0.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 148.7, 144.4, 141.4, 136.3, 135.0, 129.7, 129.6, 127.8, 127.6, 122.9, 122.4, 109.3, 102.3, 100.8, 71.8, 71.4, 59.6, 43.4, 39.7, 39.2, 27.7, 25.7, 25.5, 21.6, 18.0, -4.6, -4.8; MS (+FAB) *m/z* (%) $[M]^+$: 628 (8), 514 (13), 343 (17), 256 (10), 228 (10), 215 (19), 165 (36), 73(100); HRMS (+FAB) calcd for $C_{32}H_{44}NO_8SSi$ $[M+1]^+$: 630.2557, found 630.2492; Anal. calcd for $C_{32}H_{43}NO_8SSi$: C, 61.02; H, 6.88. Found C, 61.28, H, 7.02.

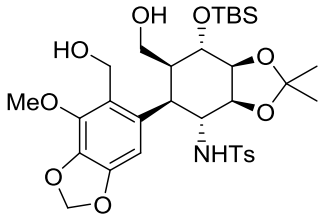
_N_-((1R,2S,3S,4S,4aR,11bR)-4-{[tert-butyl(dimethyl)silyl]oxy}-3,3-dimethoxy-7-methoxy-1,2,3,4,4a,11b-hexahydrophenanthro[2,3-d][1,3]dioxol-1-yl)benzenesulfonamide. (480)



Olefin **481** (0.100 g, 0.561 mmol), quinoline (15 mg, 0.12 mmol) and silica gel (500 mg), which has been activated in advance by heating under vacuum for 24 h at 150 °C, was charged into flask and suspended in CH_2Cl_2 (10 mL), The solvent was removed *in vacuo* and the flask containing silica gel supporting the adsorbed reactants was heated at 120 °C under nitrogen atmosphere and stirred for 36 h. After this time the reaction mixture was purified directly by column chromatography (hexanes/EtOAc 4:1) to give olefin **480** as a clear and colorless oil (0.074 g, 74%).

R_f 0.45 (hexanes: EtOAc 2:1); $[\alpha]^{24}_D = -25.1$ (c = 1.0, $CHCl_3$); IR (KBr, cm^{-1}) ν 3275, 2983, 2953, 2932, 2889, 2857, 1633, 1614, 1599, 1479, 1384, 1361, 1331, 1221, 1158, 1092, 841; ^1H NMR ($CDCl_3$, 300MHz) δ 7.43 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.1 Hz, 2H), 6.65 (dd, J = 9.8, 3.4 Hz, 1H), 6.22 (s, 1H), 5.92 (d, J = 1.5Hz, 1H), 5.82 (d, J = 1.5Hz, 1H), 5.74 (dd, J = 9.8, 1.5 Hz, 1H), 4.59 (d, J = 8.8Hz, 1H), 4.28 (m, 1H), 4.11 (s, 1H), 4.02-3.99 (m, 1H) 3.97 (s, 3H), 3.80-3.70 (m, 1H), 2.79-2.78 (m, 1H), 2.61-2.56(m, 1H), 2.41 (s, 3H), 1.73 (s, 1H), 1.45 (s, 3H), 1.34 (s, 3H), 0.88 (s, 9H), 0.10 (s, 3H), 0.05 (s, 3H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 147.5, 142.1, 139.5, 138.6, 135.5, 129.4, 129.8, 126.8, 125.3, 120.5, 119.7, 109.2, 104.8, 100.7, 79.1, 78.3, 70.3, 59.7, 53.7, 42.4, 39.1, 27.8, 26.3, 25.7, 21.5, 18.0, −5.03, −5.06. MS (+FAB) *m/z* (%): $[M]^+$ 629 (3), 129 (13), 111 (12), 99 (13), 57 (100); HRMS (+FAB) calcd for $C_{32}H_{43}NO_8SSi$ $[M]^+$: 629.2479; found, 629.2472.

N-[(3aS,4R,5R,6S,7S,7aS)-7-{[tert-butyl(dimethyl)silyl]oxy}-6,6'-bis(hydroxymethyl)-7'-methoxy-2,2-dimethyl-3,4,5,6,7,7a-hexahydro-5,5'-bi-1,3-benzodioxol-4-yl]-4-methylbenzenesulfonamide (495)

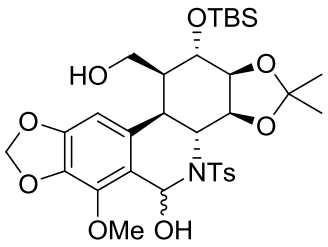


To a solution of **480** (0.254 g, 0.404 mmol) in MeOH (50 mL) a few crystals of Sudan Red 7B were added. The solution was cooled down to −80 °C and oxygen-ozone mixture was bubbled through until the disappearance of the pink color. The consumption of starting material was also checked by TLC. A stream of nitrogen was bubbled through

reaction mixture for 5 min. sodium borohydride (0.250 g, 6.67 mmol) was slowly added and reaction mixture was gradually warmed from −80 °C to room temperature. The solvent was removed *in vacuo* and the residue was redissolved in CH_2Cl_2 (50mL), neutralized by 10% citric acid and washed with water (50 mL). The organic phase was separated, dried over Na_2SO_4, filtered and evaporated. The crude product was subjected to column chromatography (hexanes/EtOAc 1:1) to yield **495** as white crystalline solid (0.1643 g, 61%) and **496** as mixture of anomers (80 mg, 30%).

R_f 0.45 (hexanes/EtOAc 1:1); mp 121-123 °C ($CHCl_3$); $[\alpha]^{20}_D$ − 30.8 (*c* = 1.09, $CHCl_3$); IR (KBr, cm^{-1}) ν 3472, 3386, 3172, 2927, 2855, 1622, 1482, 1385, 1332, 1255, 1220, 1158, 1095, 1057, 837; ^1H NMR ($CDCl_3$, 600MHz) δ 7.51 (d, *J* = 7.8 Hz, 2H), 7.13 (d, *J* = 7.8 Hz, 2H), 6.57 (s, 1H), 5.96 (s, 1H), 5.90 (s, 1H), 5.44 (d, *J* = 7.0 Hz, 1H), 4.77 (d, *J* = 11.8 Hz, 1H), 4.42 (d, *J* = 11.9 Hz, 1H), 4.27-4.24 (m, 1H) 4.17-4.10 (m, 2H), 4.00 (s, 3H), 3.95-3.86 (m, 1H), 3.70 (dd, *J* =11.9, 6.1 Hz, 1H), 3.59 (dd, *J* =11.8, 6.3 Hz, 1H), 3.37 (dd, *J* = 11.8, 3.9 Hz, 1H), 2.92 (br s, 2H), 2.39 (s, 3H), 2.00-1.96 (m, 1H), 1.56 (s, 3H), 1.32 (s, 3H), 0.92 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$, 150MHz): δ 149.1, 142.1, 141.6, 139.2, 135.1, 132.4, 128.8, 126.8, 124.6, 109.7, 103.5, 101.0, 79.9, 79.0, 71.5, 61.3, 60.0, 57.0, 55.2, 47.3, 38.2, 27.4, 25.9, 25.8, 21.5, 18.0, 21.6, 21.0, 18.0, −4.8, −5.0; MS (+FAB) m/z (%): 664 $[M-H]^+$ (6), 648 (7), 372 (11), 302 (11), 254 (21), 248 (12), 73 (100), HRMS(+EI) calcd for $C_{32}H_{47}NO_{10}SSi$ $[M]^+$: 665.2690; found 665.2803; Anal. calcd for $C_{32}H_{47}NO_{10}SSi$: C, 57.72; H, 7.11. Found C, 57.76; H, 6.99.

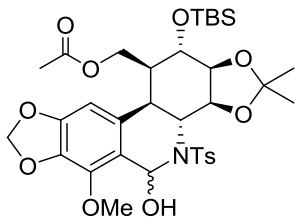
(3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-11-(hydroxymethyl)-6-methoxy-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-5-ol (496)



To a solution of alcohol **495** (0.100 g, 0.15 mmol) in CH_2Cl_2 (100 mL) was added MnO_2 (268 mg, 3 mmol). The reaction mixture was vigorously stirred until total consumption of starting material (TLC). The reaction mixture was filtered through a plug of Celite® and washed with CH_2Cl_2 (3 × 100 mL). Solvent was removed *in vacuo*, affording **496** as white solid (87mg, 87 %, mixture of anomers).

R_f 0.5 and 0.65 (hexanes:EtOAc 1:1); mp 106-116 °C ($CHCl_3$); IR (KBr, cm^{-1}) ν 3452, 2985, 2954, 2931, 2894, 2857, 1624, 1483, 1384, 1341, 1251, 1163, 1077, 839; (NMR of the major anomer) ^{1}H NMR ($CDCl_3$, 300MHz) δ 7.55 (d, *J* = 8.1 Hz, 2H), 7.03 (d, *J* = 8.1 Hz, 2H), 6.65 (s, 1H), 6.08 (s, 1H), 5.92-5.91 (m, 2H), 5.26 (dd, *J* = 9.9, 5.4 Hz,1H), 4.38 (t, *J* = 3.0 Hz, 1H) 4.25-4.21 (m, 2H), 4.11 (s, 3H), 3.74 (dd, *J* = 11.0, 7.9 Hz, 1H), 3.37 (dd, *J* = 11.0, 3.6 Hz, 1H), 2.87 (dd, *J* = 12.9, 5.1 Hz, 1H), 2.33 (s, 3H), 2.16 (br s, 1H), 2.15 (br s, 1H), 2.06 (s, 1H), 1.44 (s, 3H), 1.37 (s, 3H), 0.92 (s, 9H), 0.15 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$, 150MHz) δ 150.0, 142.9, 140.5, 138.3, 134.0, 130.0, 128.9, 127.2, 121.3, 109.4, 101.0, 100.2, 78.9, 76.4, 73.5, 68.1, 59.9, 59.3, 53.1, 48.1, 34.4, 27.9, 26.2, 25.7, 21.4, 17.9, −4.8, −5.1; MS (+EI) m/z (%): $[M\text{-}Ts\text{-}H_2O]^+$ 491 (15), 432 (13), 302 (22.2), 302 (10.5), 247 (31.1), 246 (19.9), 43 (100); HRMS (+EI) calcd for $C_{32}H_{45}NO_{10}SSi$ $[M]^+$: 663.2533; found 663.2549; Anal. Calcd for $C_{32}H_{45}NO_{10}SSi$: C, 57.90; H, 6.83. Found C, 58.02; H, 7.03.

{(3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-5-hydroxy-6-methoxy-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-11-yl}methyl acetate (497)

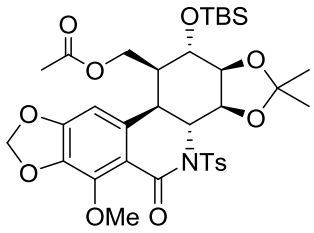


Pyridine (0.050 g, 0.63 mmol) was added to a solution of hemiacetal **496** (0.048 g, 0.072 mmol) in CH_2Cl_2 (3 mL), followed by addition of acetic anhydride (0.0295 g, 0.29 mmol). The reaction mixture was stirred until consumption of starting material (TLC). The reaction mixture was quenched with water (5 mL) and extracted by CH_2Cl_2 (3 × 4 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated. The crude product was subjected to column chromatography (eluent hexanes/EtOAc 4:1) affording **497** as colourless oil (0.043 g, 87 % mixture of anomers).

R_f 0.9 and 0.8 (Hexanes/EtOAc 1:1); IR (KBr, cm^{-1}) ν 3462, 2984, 2953, 2931, 2896, 2857, 1743, 1624, 1481, 1371, 1342, 1330, 1250, 1222, 1164, 1077, 840; (NMR of the major anomer) ^{1}H NMR ($CDCl_3$, 600MHz) δ 7.56 (d, *J* = 8.2 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 6.66 (s, 1H), 6.012 (s, 1H), 5.94 (m, 2H), 5.31 (dd, *J* = 10.0, 5.1 Hz, 1H), 4.37 (m, 1H) 4.22-4.21 (m, 1H), 4.08 (s, 3H), 3.96 (s, 1H), 3.91 (dd, *J* = 11.4, 4.4 Hz, 1H), 3.05 (s, 1H), 2.91 (dd, *J* = 13.2, 4.5 Hz, 1H), 2.35 (s, 3H), 2.15 (s, 1H), 1.93 (s, 3H), 1.47 (s, 3H), 1.38 (s, 3H), 0.93 (s, 9H), 0.15 (s, 3H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$, 150MHz): δ 170.8 150.0, 143.0, 140.6, 138.5, 134.1, 129.8, 128.9, 127.2, 121.3, 109.3, 101.0, 100.1, 78.9, 76.3, 73.3, 67.2, 61.2, 59.9, 53.2, 45.6, 33.8, 29.7, 26.3, 25.7, 25.5,

21.4, 20.8, 17.9 −5.0, −5.2; MS (+FAB) m/z (%): $[M-H_2O]^+$ 688 (47), 230 (12), 117 (17), 302 (11), 247 (31), 117 (17), 73 (100); HRMS (+FAB) calcd for $C_{34}H_{46}NO_{10}SSi$ [M - $H_2O]^+$: 688.2612; found 688.2642; Anal. calcd for $C_{34}H_{47}NO_{11}SSi$: C, 57.85; H, 6.71. Found C, 57.80; H, 6.67.

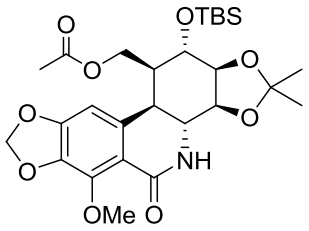
{(3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-6-methoxy-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-11-yl}methyl acetate (498)



A pre-dried solution of *N*-methylmorpholine-*N*-oxide (0.100 g, 0.85 mmol) in CH_2Cl_2 (20 mL) was added to hemiaminal **497** (0.030 g, 0.042 mmol) in CH_2Cl_2 (10 mL), followed by activated crushed molecular sieves (0.5 g, 4 Å). After stirring for 15 min, a few crystals of tetrapropylammonium perruthenate were added and reaction was stirred until consumption of starting material (TLC). The reaction mixture was filtered through a plug of Celite® and washed with CH_2Cl_2 (3 × 50 mL). The combined organic phases were evaporated and subjected to column chromatography (hexanes/EtOAc 2:1) affording **498** as colorless oil (0.025 g, 84 %)

R_f 0.65 (eluent hexanes/EtOAc 1:1); $[\alpha]^{24}_D$ + 41.4 (c = 0.9, $CHCl_3$); IR (KBr, cm^{-1}): ν 3450, 2984, 2953, 2930, 2857, 1742, 1710, 1614, 1484, 1360, 1253, 1169, 1090, 1021, 839; ^1H NMR ($CDCl_3$, 300MHz) δ 8.20 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 8.3 Hz, 2H), 6.73 (s, 1H), 6.06 (s, 1H), 6.02 (s, 1H), 4.83 (dd, J = 7.9, 5.5 Hz, 1H), 4.46 (s, 1H), 4.26 (t, J = 11.1, 1H), 4.17-4.13 (m, 1H), 4.12-4.08 (m, 1H), 4.04 (s, 3H), 4.00-3.95 (m, 1H), 3.47 (dd, J = 13.1, 3.3 Hz, 1H), 2.66-2.63 (m, 1H), 2.43 (s, 3H), 2.08 (s, 1H), 1.48 (s, 3H), 1.33 (s, 3H), 0.89 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H); ^{13}C NMR ($CDCl_3$, 150MHz) δ 170.8, 163.7, 153.4, 144.8, 143.4, 138.8, 136.7, 136.1, 128.9, 128.7, 126.8, 117.8, 109.1, 102.0, 100.1, 79.0, 76.3, 66.9, 60.9, 60.7, 60.4, 59.7, 43.4, 36.5, 27.9, 26.1, 25.7, 21.6, 20.9, 18.0, −4.9, −5.1; MS (+EI) *m/z* (%): 639 (3.1), 549 (6.6), 492 (3.4), 434 (2.9), 374 (3.9), 43 (100); HRMS (+EI) Calcd for $C_{33}H_{42}NO_{11}SSi^+$ $[M-CH_3]^+$: 688.2248; found 688.2436.

((3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-6-methoxy-2,2-dimethyl-5-oxo-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-11-yl)methyl acetate (499)

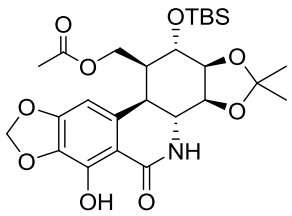


To a solution of **498** (0.064 mg, 0.09 mmol) in dry DME (7 mL) at −50 °C was added dropwise a solution of Na/naphthalene in DME (0.5 M), until a light green color persisted and total consumption of starting material was observed (TLC). The solution was stirred for 10 minutes before the reaction was quenched with NH_4Cl (satd. aq., 1 mL). The

reaction was warmed to room temperature and extracted with CH_2Cl_2 (6 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The products **499** and **479** were isolated by column chromatography (gradient hexanes/EtOAc 2:1 → 1:1) as clear and colorless oil **499** (0.030 g, 62%) and white crystalline solid **479** (0.002 g; 5%).

R_f 0.28 (hexanes: EtOAc 1:1); $[\alpha]^{20}_D$ + 32.4 (*c* = 1.0, $CHCl_3$); IR (KBr, cm^{-1}) ν 3417, 3228, 3109, 2987, 2953, 2932, 2897, 2858, 1743, 1676, 1617, 1481, 1385, 1366, 1339, 1250, 1222, 1169, 1088, 1071, 1057, 1033, 840; ^1H NMR ($CDCl_3$, 600MHz) δ 6.69 (s, 1H), 6.06 (s, 1 H), 6.01 (s, 1H), 5.92 (s, 1H), 4.57 (s, 1H), 4.23 (d, *J* = 7.5 Hz, 1H), 4.19-4.18 (m, 1H), 4.16-4.14 (m, 1H), 4.07 (s, 3H), 3.42 (dd, *J* =13.8, 8.3 Hz, 1H), 3.31 (dd, *J* =13.8, 3.6 Hz, 1H), 2.66-2.65 (m, 1H), 2.11 (s, 3H), 1.45 (s, 3H), 1.39 (s, 3H), 0.91 (s, 9H), 0.16 (s, 3H), 0.15 (s, 3H); ^{13}C NMR ($CDCl_3$, 150MHz) δ 170.9, 163.5, 152.4, 145.4, 137.5, 135.5, 116.2, 109.9, 101.8, 99.9, 78.3, 77.8, 67.0, 61.1, 60.9, 52.5, 41.9, 35.1, 28.2, 26.1, 25.69, 25.65, 20.9, 18.0, −5.0, −5.1; MS (+FAB) m/z (%): 552 (12), 551 (36), 550 $[M+1]^+$ (100), 246 (10), 220 (11), 117 (101); HRMS (+FAB) calcd for $C_{27}H_{40}NO_9Si$ $[M+1]^+$: 550.2472; found 550.2459.

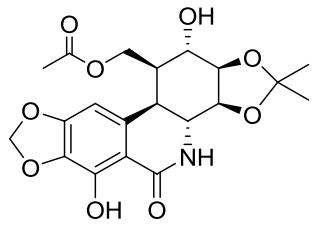
((3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-6-hydroxy-2,2-dimethyl-5-oxo-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-11-yl)methyl acetate (500)



To a solution of **499** (0.039mg, 0.071 mmol) in DMF (5 mL) was added LiCl (0.05 mg, 1.2 mmol), followed by three cycles freeze-pump-thaw. The reaction mixture was heated to 120 °C for 2.5 h. The reaction mixture was cooled to room temperature, diluted with water (100 ml) and extracted with diethyl ether (10 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The product was isolated by column chromatography (hexanes:EtOAc, 3:1) as a clear and colorless oil which solidifies after drying (25.7 mg, 68%).

R_f 0.8 (hexanes: EtOAc 1:1); mp 68-69° C ($CHCl_3$); $[\alpha]^{20}_D$ − 35.2 (*c* = 1.0, $CHCl_3$); IR (KBr, cm^{-1}) ν 3402, 3348, 3285, 3212, 3087, 2987, 2953, 2933, 2899, 2859, 2795, 1743, 1627, 1601, 1464, 1389, 1366 , 1353, 1341, 1301, 1250, 1226, 1171, 1081, 1032, 940, 838, 778; ^{1}H NMR ($CDCl_3$, 600MHz) δ 12.68 (s, 1H), 6.53 (s, 1H), 6.26 (s, 1H), 6.07 (s, 2H), 4.57 (s, 1H), 4.30 (dd, *J* = 11.1, 3.3 Hz, 1H), 4.22 (t, *J* = 11.0 Hz, 1H) 4.21-4.18 (m, 2H), 3.50 (dd, *J* = 14.4, 7.8 Hz, 1H), 3.36 (dd, *J* = 14.4, 3.7 Hz, 1H), 2.70-2.69 (m, 1H), 2.11 (s, 3H), 1.49 (s, 3H), 1.41 (s, 3H), 0.91 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 150MHz) δ 170.8, 169.9, 153.1, 146.9, 133.9, 133.0, 110.0, 107.3, 102.3, 97.3, 78.4, 77.6, 67.0, 61.10, 53.2, 41.7, 33.9, 28.3, 26.0, 25.7, 20.9, 18.0, −5.0, −5.1; MS (+EI) m/z (%): 536 $[M+1]^+$(9), 535 (25), 360 (17), 256 (11), 231 (10), 218 (19), 205 (21), 149 (25), 43 (100); HRMS (+EI) calcd for $C_{26}H_{37}NO_9Si$ $[M]^+$: 535.2238; found 535.2248.

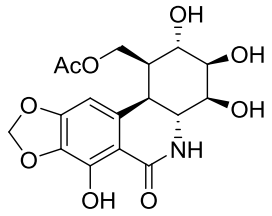
[(3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*R*)-6,12-dihydroxy-2,2-dimethyl-5-oxo-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-11-yl]methyl acetate (501)



The compound **500** (0.052 g, 0.097 mmol) was taken up in THF (2.5 mL) and cooled to 0 °C. A solution of TBAF in THF (1 M, 0.107 mL, 0.107 mmol) was added dropwise over 1 min. The reaction mixture was stirred until total consumption of starting material was observed (TLC). The reaction mixture was quenched with water (5 ml) and extracted with CH_2Cl_2 (6 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated. The final product was isolated by column chromatography (hexanes/EtOAc 1:1) as a white crystalline solid (38 mg, 95%).

R_f 0.3 (hexanes/EtOAc 1:1); mp > 200 °C (CH_2Cl_2 - CH_3OH); $[\alpha]^{24}_D$ + 6.2 (*c* = 0.48, DMSO); IR (KBr, cm^{-1}) ν 3449, 3270, 2988, 2911, 1743, 1672, 1625, 1600, 1466,1443, 1357, 1307, 1245, 1231, 1166, 1087, 1071, 1032, 844; ^{1}H NMR (DMSO-d6, 600MHz) δ 13.35 (s, 1H), 8.55 (s, 1H), 6.61 (s, 1H) 6.08 (s, 1H), 6.06 (s, 1H), 5.50 (d, *J* = 3.6 Hz, 1H) , 4.34 (br. s, 1H), 4.25 (d, *J* = 4.8 Hz, 1H), 4.20 (dd, *J* = 11.4, 3.6 Hz, 1H), 4.16 - 4.15 (m, 1H), 4.13 (d, *J* = 11.4 Hz, 1H) 3.51 (dd, *J* = 15.0, 8.4 Hz, 1H), 3.19 (dd, *J* = 14.4, 3.6 Hz, 1H), , 2.03 (s, 3H), 1.41 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (DMSO-d_6, 150MHz) δ 170.9, 169.8, 152.7, 146.3, 135.0, 132.6, 109.0, 107.7, 102.4, 97.6, 77.9, 76.9, 65.2, 61.2, 53.4, , 34.3, 28.3, 26.4, 21.2; MS (+EI) m/z (%): 422 (24) [M+1], 421 (100) [M^+], 248 (34), 247 (60), 232 (27), 231 (32), 218 (11), 206 (17), 145 (14); HRMS Calcd for $C_{20}H_{23}NO_9$ [M]$^+$: 421.13728; found 421.13792; Anal. calcd for $C_{20}H_{23}NO_9$: C, 57.00; H, 5.50. Found C, 57.18, H, 5.48.

[(1*S*,2*S*,3*R*,4*S*,4a*R*,11b*R*)-2,3,4,7-tetrahydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro[1,3]dioxolo[4,5-*j*]phenanthridin-1-yl]methyl acetate (5b)

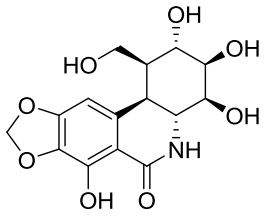


The compound **501** (0.038 g, 0.09 mmol) was taken up in CH_2Cl_2:CH_3OH mixture (1:1, 4 mL) and cooled to 0 ˚C. Trifluoroacetic acid (2 mL) was added dropwise and the reaction mixture was stirred until consumption of starting material was observed (TLC). The reaction mixture was dried *in vacuo*, triturated with CH_2Cl_2 (3 × 15 mL) and finally dried under high-vac. The final product was isolated by column chromatography on silica gel (deactivated by 10% w/w of water, eluent CH_2Cl_2:CH_3OH 10:1) as a white crystalline solid (0.031 g, 90%).

R_f 0.3 (CH_2Cl_2:CH_3OH 10:1); mp > 200 °C (CH_2Cl_2 - CH_3OH); $[\alpha]^{24}_D$ + 36.8 (*c* = 0.2,THF); IR (KBr, cm^{-1}) ν 3459, 3287, 3214, 2991, 2923, 1750, 1709, 1670, 1628, 1595, 1466, 1436, 1384, 1342, 1264, 1227, 1196, 1090, 1070, 1034; ^{1}H NMR (DMSO-d6, 600MHz) δ 13.26 (s, 1H), 7.40 (s, 1H), 6.59 (s, 1H), 6.08 (s, 1H), 6.06 (s, 1H), 5.17 (m, 1H), 5.10-5.09 (m, 2H), 4.40 (m, 1H), 4.16 (dd, *J* = 10.9, 3.5 Hz, 1H), 4.11 (s, 1H), 3.84 (m, 1H), 3.71 (m, 1H), 3.52 (dd, *J* = 13.6, 9.8 Hz, 1 H), 3.26 (m, 1H), 2.66 (m, 1H), 2.03 (s, 3H); ^{13}C NMR (DMSO-d_6, 150MHz) δ 171.0, 169.9, 152.8, 146.3, 135.7, 132.5, 107.5, 102.4, 97.8, 73.1, 71.2, 68.9, 61.8, 51.6, 40.5, 36.5, 21.3; MS (+EI) *m/z* (%): 381 $[M]^+$ (12), 321 (16), 279 (12), 277 (99), 276 (11), 256 (12), 247 (11), 205 (13), 201 (16),

199 (12), 185 (13), 183 (11), 179 (21), 167 (14), 149 (37), 129 (12), 123 (11), 69 (100); HRMS (+EI) calcd for $C_{17}H_{19}NO_9$: 381.1060; found 381.1055;

(1*S*,2*S*,3*R*,4*S*,4a*R*,11b*R*)-2,3,4,7-tetrahydroxy-1-(hydroxymethyl)-1,3,4,4a,5,11b-hexahydro[1,3]dioxolo[4,5-*j*]phenanthridin-6(2*H*)-one (5a)

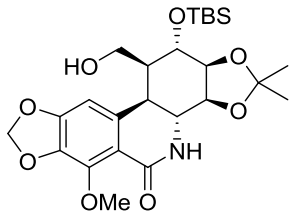


The compound **501** (48 mg, 0.11 mmol) was taken up in CH_2Cl_2-CH_3OH mixture (1:1, 4 mL) and 2 drops of concentrated HCl were added. The reaction was stirred until total consumption of starting material was observed (TLC). The reaction mixture was neutralized by dropwise addition of saturated solution of $NaHCO_3$ and evaporated to dryness. The final product was isolated by column chromatography on silica gel (deactivated by 10% of water, eluent CH_2Cl_2:CH_3OH 5:1) as white crystalline compound (36 mg, 92%).

mp >200 °C (CH_2Cl_2-CH_3OH); R_f 0.4 (CH_2Cl_2: CH_3OH 5:1); $[\alpha]^{20}_D$= + 48.0 (*c* = 0.5, abs DMSO); IR (KBr, cm^{-1}) ν 3386, 2917, 1670, 162, 1598, 1466, 1439, 1384, 1352, 1304, 1228, , 1089, 1077, 1064, 1032; ^{1}H NMR (DMSO-d6, 300MHz) δ 13.25 (s, 1H), 7.31 (s, 1H), 6.55 (s, 1H), 6.07 (s, 1H), 6.06 (s, 1H), 5.02 (m, 3H), 4.47 (dd, *J* =6.4, 4.0 Hz, 1H), 4.18 (m, 1H), 3.91 (m, 1H), 3.82 (m, 1H), 3.67 (m, 1H), 3.43 (m, 1H), 3.14 (m, 1H), 2.37

(br. s, 1H); ^{13}C NMR (DMSO-d6, 150MHz) δ 169.9, 152.8, 146.1, 136.7, 132.2, 107.4, 102.4, 97.9, 73.2, 71.4, 69.5, 57.7, 51.8, 44.3, 36.9; MS (+EI) m/z (%) 339 $[M]^+$: (0.4), 85 (39), 84 (80), 83 (57), 68 (13), 66 (100); HRMS (+EI) calcd for $C_{15}H_{17}NO_8$: 339.0954; found 339.0925.

(3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-11-(hydroxymethyl)-6-methoxy-2,2-dimethyl-3b,4,10b,11,12,12a-hexahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-5(3a*H*)-one (479)

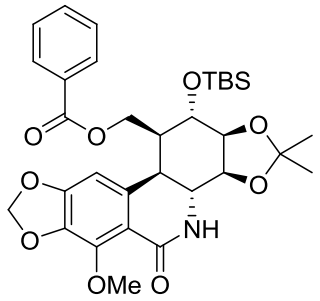


To a solution of **499** (0.170 g, 0.309 mmol) in methanol (5 mL) was added solution of NaOH (aq. 40%, 0.5 mL) and stirred until total consumption of starting material was observed (TLC). The reaction was quenched with solution of NH_4Cl (sat. aq., 1 mL). The mixture was evaporated and the residue was extracted with CH_2Cl_2 (6 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The final product was isolated by column chromatography (gradient hexanes:EtOAc 2:1 to 1:1) as a white crystalline solid (0.125 mg; 80%).

R_f 0.2 (hexanes/EtOAc 1:1); mp 148-149 °C ($CHCl_3$); $[\alpha]_D^{20}$ +32.6 (*c* = 0.63, $CHCl_3$); IR (KBr, cm^{-1}) ν 3416, 2987, 2952, 2932, 2893, 2857, 1660, 1618, 1501, 1481, 1439, 1384, 1350, 1295, 1250, 1220, 1169, 1083, 1056, 841; ^{1}H NMR ($CDCl_3$, 600MHz) δ 6.61 (s,

1H) 6.05 (s, 1H), 6.00 (s, 1H), 5.86 (s, 1H), 4.68(s, 1H), 4.20 (d, *J* =4.5 Hz, 1H), 4.14 (dd, *J* =8.2, 5.0 Hz, 1H), 4.08 (s, 3H), 3.95 (dt, *J* = 10.1, 6.0, 1H), 3.67-3.66 (m, 1H), 3.49 (dd, *J* =13.8, 8.4 Hz, 1H), 3.28 (dd, *J* =13.8, 3.7 Hz, 1H), 2.53-2.52 (m, 1H), 1.91 (s, 1H), 1.46 (s, 3H), 1.40 (s, 3H), 0.91 (s, 9H), 0.18 (s, 6H); ^{13}C NMR ($CDCl_3$, 150MHz) δ 163.6, 152.2, 145.4, 137.3, 136.3, 116.3, 101.7, 99.8, 78.3, 78.0, 67.4, 60.9, 58.8, 52.7, 45.2, 35.3, 28.1, 26.2, 25.7, 17.9, −4.92, −4.94; MS (+FAB) m/z (%): 508 (14) [M+1], 507 (37) [M^+], 450 (16), 449 (10), 434 (17), 433 (16), 392 (22), 374 (12), 345 (12), 261(100); HRMS (+FAB) Calc for $C_{25}H_{37}NO_8Si$ [M]$^+$: 507.2289; found: 507.2287.

((3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-6-methoxy-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-11-yl)methyl benzoate (503)

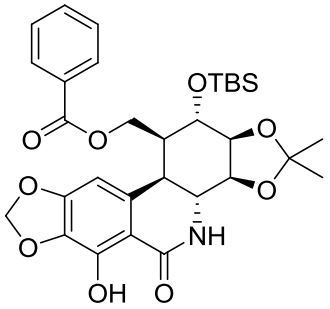


To a solution of **479** (0.121 g, 0.24 mmol) in CH_2Cl_2 (25 mL) was added triethylamine (0.04 mL, 0.48 mmol) at 0 °C followed by benzoyl chloride (0.03 mL, 0.26 mmol) and crystal of DMAP. The reaction mixture was stirred at 0 ˚C until total consumption of starting material was observed (TLC). The reaction mixture was quenched with distilled water (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with solution of citric acid (10%, 10 mL), dried over sodium sulfate,

filtered, and concentrated. The final product was isolated by column chromatography (gradient hexanes/EtOAc 2:1→1:1) as white crystalline powder (0.094 g, 64%).

R_f 0.4 (hexanes:EtOAc 1:1); mp 90-92 °C ($CHCl_3$); $[\alpha]_D^{20}$ − 15.1 (*c* = 1, $CHCl_3$); IR (KBr, cm^{-1}) ν 3467, 3416, 3387, 3069, 2986, 2952, 2932, 2896, 2857, 1722, 1674, 1616, 1502, 1481, 1453, 1385, 1339, 1273, 1221, 1093, 1071, 1028, 838; ^1H NMR ($CDCl_3$, 300MHz) δ 8.06 (d, *J* = 7.2 Hz, 2H), 7.63-7.58 (m, 1H), 7.48 (t, *J* = 7.5 Hz, 2H), 6.74 (s, 1H), 6.01-6.02 (m, 3H), 4.68 (s, 1H), 4.53-4.45 (m, 2H), 4.20-4.16 (m, 2H), 4.07 (s, 3H), 3.53 (dd, *J* = 13.8, 7.5 Hz, 1H), 3.40-3.35 (m, 1H), 2.86-2.83 (m, 1H), 1.50 (s, 3H), 1.40 (s, 3H), 0.90 (s, 9H), 0.18 (s, 6H); ^{13}C NMR ($CDCl_3$, 75MHz) δ 166.4, 163.6,152.4, 145.4, 135.6, 133.2, 129.8, 129.6, 128.4, 116.1, 110.0, 101.7, 99.9, 78.3, 77.9, 67.2, 61.6, 60.9, 52.5, 42.1, 35.2, 28.2, 26.1, 25.6, 17.9, −4.9, −5.0; MS (+FAB) m/z (%): 614 (12) [M+2$^+$], 613 (36), 612 (89), 179 (12), 105 (100); HRMS Calc for $C_{32}H_{42}NO_9Si^+$ $[M]^+$: 612.2629; found: 612.2653.

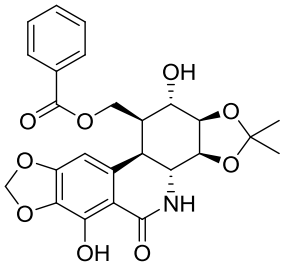
((3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-6-hydroxy-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-11-yl)methyl benzoate (504)



To a solution of **503** (0.0743 g, 0.122 mmol) in dry DMF (5 mL) was added LiCl (0.050 g, 1.2 mmol), followed by three cycles of freeze-pump-thaw. The reaction mixture was heated to 120 °C for 3.5 h. The reaction was then cooled to room temperature, diluted with distilled water (50 ml) and extracted with diethyl ether (6 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The product was isolated by column chromatography (hexanes/EtOAc 2:1) as a white crystalline solid (0.060 g, 83%).

R_f 0.8 (hexanes:EtOAc 1:1); mp 141-145 °C ($CHCl_3$); $[\alpha]^{20}_D$ − 63.0 (*c* = 1.0, $CHCl_3$); IR (KBr, cm^{-1}) ν 3449, 2953, 2931, 2901, 2858, 1721, 1674, 1655, 1637, 1627, 1603, 1461, 1385, 1351, 1341, 1304, 1271, 1219, 1113, 1081, 837; ^{1}H NMR ($CDCl_3$, 600MHz) δ 12.70 (s, 1H), 8.07 (d, *J* = 8.0 Hz, 2H), 7.62-7.60 (m, 1H), 7.49-7.47 (m, 2H), 6.59 (s, 1H), 6.11 (s, 1H), 6.06 (s, 1H), 6.02 (s,1H), 4.68 (s, 1H), 4.55-4.54 (m, 2H), 4.23-4.21 (m, 2H), 3.63-3.59 (m, 1H), 3.43 (dd, *J* =14.4, 3.5 Hz, 1H), 2.89 (s br, 1H), 1.53 (s, 3H), 1.42 (s, 3H), 0.91 (s, 9H), 0.16-0.15 (m, 6H); ^{13}C NMR ($CDCl_3$, 150MHz) δ 170.0, 166.4, 153.2, 146.9, 134.0, 133.2, 133.1, 129.8, 129.6, 129.4, 110.1, 107.3, 102.3, 97.3, 78.4, 77.7, 67.2, 61.6, 53.2, 41.8, 34.0, 30.3, 28.4, 26.1, 25.7, 17.9, −4.9, −5.0; MS (+FAB) m/z (%): 599 (11) $[M+2]^+$, 598 (29), 597 (8), 596 (4), 179 (11), 73 (100); HRMS (+FAB) calcd. for $C_{31}H_{40}N_1O_9Si^+$ $[M+1]^+$: 598.2472; found 598.2446;

[(3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*R*)-6,12-dihydroxy-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-11-yl]methyl benzoate (505)

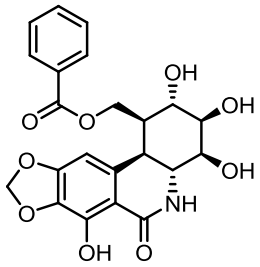


The compound **504** (0.042 mg, 0.07 mmol) was taken up in THF (2.5 mL) and cooled to 0 °C. A solution of TBAF in THF (1 M, 0.077 mL, 0.077 mmol) was added dropwise and the reaction mixture was stirred until total consumption of starting material was observed (TLC). The reaction mixture was quenched by distilled water (5 ml) and extracted with CH_2Cl_2 (6 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered, and evaporated. The final product was isolated by column chromatography (hexanes/EtOAc 1:1) as white crystalline solid (0.0272 g, 80%).

R_f 0.3 (hexanes/EtOAc 1:1); mp > 200 °C (THF); $[\alpha]_D^{21}$ −40.1 (*c* = 1.0, THF); IR (KBr, cm^{-1}) ν 3446, 3255, 2986, 2930, 2905, 2854, 1720, 1672, 1626, 1601, 1466, 1384, 1356, 1343, 1311, 1222, 1166, 1088, 1071, 1027, 713; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 13.36 (s, 1H), 8.57 (s, 1H), 7.97-7.96 (m, 2H), 7.69-7.67 (m, 1H), 7.56-7.53 (m, 2H), 6.74 (s, 1H), 6.07 (s, 1H), 5.99 (s, 1H), 5.56 (dd, *J* = 4.3 Hz, 1H), 4.53 (dd, *J* = 11.3, 4.3 Hz, 1H), 4.46-4.45 (m, 1H), 4.43-4.39 (m, 1H), 4.29 (d, *J* = 5.0 Hz, 1H), 4.19 (dd, *J* = 8.3, 5.3 Hz, 1H), 3.63 (dd, *J* = 14.4, 8.4 Hz, 1H), 3.26 (dd, *J* = 14.4, 3.6 Hz, 1H), 2.97-2.95 (m, 1H), 1.45 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (DMSO-d_6, 150MHz) δ 169.9, 166.1, 152.7, 146.3, 135.0, 133.9, 132.5, 130.0, 129.7, 129.2, 109.0, 107.7, 102.3, 97.9, 78.0, 77.1, 65.5, 62.0, 53.4, 34.3, 30.3, 28.3, 26.4; MS (+FAB) *m/z* (%): 484 (4) $[M+1]^+$, 483 (26) $[M]^+$, 248

(13), 247 (59), 232 (13), 231 (28), 205 (11), 122 (16), 105 (100); HRMS (+FAB) Calc. for $C_{25}H_{25}NO_9$ $[M]^+$: 483.1529; found: 483.1532.

[(1*S*,2*S*,3*R*,4*S*,4a*R*,11b*R*)-2,3,4,7-tetrahydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro[1,3]dioxolo[4,5-*j*]phenanthridin-1-yl]methyl benzoate (5c)

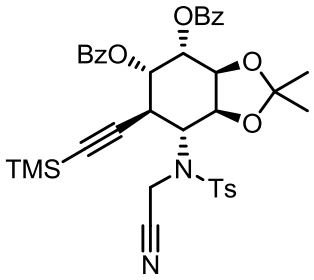


The compound **505** (32 mg, 0.066 mmol) was taken up in a mixture of CH_2Cl_2:CH_3OH (1:1, 4 mL) and cooled to 0 ˚C. Trifluoroacetic acid (1 mL) was added dropwise and the reaction was stirred until total consumption of starting material was observed (TLC). The reaction mixture was dried *in vacuo*, triturated with CH_2Cl_2 (3 × 15 ml) and finally dried under high-vac. The final product was isolated by column chromatography on silica gel (deactivated by 10% of water, gradient CH_2Cl_2 → CH_2Cl_2/CH_3OH 50:1 → CH_2Cl_2/CH_3OH 25:1) as white crystalline solid (0.025 g, 85%).

R_f 0.6 (CH_2Cl_2:CH_3OH 10:1); mp> 200 °C (CH_2Cl_2); $[\alpha]_D^{20}$ − 24.9 (*c* = 1.0, THF); IR (KBr, cm^{-1}) ν 3423, 3386, 2956, 2921, 2852, 1716, 1672, 1627, 1600, 1466, 1384, 1363, 1340, 1278, 1095, 1072, 1038, 711; ^{1}H NMR (DMSO-d6, 600MHz) δ 13.27 (s, 1H), 8.00 (d, *J* = 7.7 Hz, 2H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.57-7.54 (m, 2H), 7.43 (s, 1H), 6.72 (s, 1H) 6.07 (s, 1H), 6.01 (s, 1H), 5.19 (m, 2H), 5.13 (m, 1H), 4.66 (t, *J* = 10.7 Hz, 1H), 4.48 (dd, *J* =10.9, 4.0 Hz, 1H), 4.24-4.23 (m, 1H), 3.88-3.87 (m, 1H), 3.75-3.73 (m, 1H), 3.62 (dd, *J* =13.6, 9.9 Hz, 1H), 3.31 (dd, *J* = 13.8, 4.2 Hz, 1H), 2.85-2.84 (m, 1H); ^{13}C NMR

(DMSO-d6, 150MHz) δ 170.0, 166.3, 152.8, 146.3, 135.8, 133.8, 132.5, 130.3, 129.7, 129.2, 107.5, 102.4, 98.0, 73.1, 71.2, 69.2, 62.5, 51.6, 36.6; MS (+FAB) *m/z* (%): 444 (4) $[M+1]^+$, 219 (12), 136 (11), 121 (11), 109 (15), 107 (17), 105 (23), 97 (18), 95 (29), 55 (100); HRMS (+FAB) calcd for $C_{22}H_{22}NO_9$ $[M+1]^+$: 444.1295; found: 444.1262.

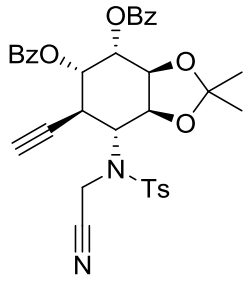
(3a*R*,4*R*,5*R*,6*S*,7*S*,7a*R*)-7-{(cyanomethyl)[(4-methylphenyl)sulfonyl]amino}-2,2-dimethyl-6-[(trimethylsilyl)ethynyl]hexahydro-1,3-benzodioxole-4,5-diyl dibenzoate (512)



The solution of acetylene **510**[92] (0.1713 mg; 0.261 mmol) in THF (40 mL) was added dropwise solution of NaHMDS in toluene (0.725 M, 0.261mmol; 0.36 mL;) at –70 °C. The reaction mixture was allowed to warm up to 0 °C over a period of 20 min and was further stirred for 10 min at this temperature. Chloroacetonitrile (33 μl; 0.522 mmol) and *n*Bu4NI (144.6 mg; 0.391 mmol) were added and solution was allowed to warm to room temperature overnight. The reaction mixture was quenched by addition of NH_4Cl (satd. aq., 10 mL) and extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, and the solvent was removed under reduced pressure. Flash column chromatography of the residue (hexanes/EtOAc 9:1) afforded tosylamide **512** (0.088 g; 48%) as colourless oil.

R_f 0.70 (hexanes/EtOAc 2:1); $[\alpha]_D^{20}$ − 108.1 (c = 1, $CHCl_3$); IR (KBr, cm^{-1}) ν 3440, 3293, 3067, 2990, 2937, 2593, 1729, 1601, 1452, 1375, 1353, 1336, 1316, 1274, 1248, 1221, 1248, 1221, 1160, 1096, 1070, 1040, 926, 890, 853, 815, 710, 666, 545, 564; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J = 7.5 Hz, 2H), 7.96 (m, 4H), 7.60 (m, 1H), 7.49 (m, 1H), 7.36 (m, 2H), 7.36 (m, 4H), 6.01 (t, J = 3.0 Hz, 1H), 5.69 (m, 1H), 4.93 (br s, 1H), 4.42 (m, 1H), 4.33 (m, 2H), 4.07 (br s, 2H), 2.43 (s, 3H), 1.68 (s, 3H), 1.39 (s, 3H), −0.02 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.1, 164.8, 144.6, 136.3, 133.7, 133.2, 129.98, 129.89, 129.88, 129.4, 129.1, 128.7, 128.3, 128.1, 114.7, 110.9, 75.1, 71.2, 68.3, 34.5, 28.3, 26.0, 21.6, −0.51; MS (+EI) *m/z* (%): 686 (2), 685 (4), 149 (12), 105 (100); HRMS (+EI) calcd for $C_{36}H_{37}N_2O_8SSi^+$ $[M\text{-}CH_3]^+$: 685.2040; found: 685.2052.

(3a*S*,4*S*,5*S*,6*R*,7*R*,7a*S*)-7-{(cyanomethyl)[(4-methylphenyl)sulfonyl]amino}-6-ethynyl-2,2-dimethylhexahydro-1,3-benzodioxole-4,5-diyl dibenzoate (471)

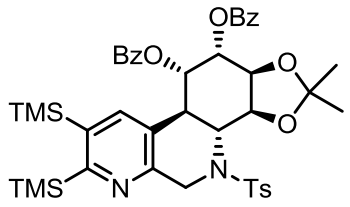


To the solution of acetylene **512** (0.093 g; 0.158 mmol) in THF (2 mL) was added dropwise solution of NaHMDS in toluene (0.75 M; 0.158mmol; 0.210 mL) at –70 °C. The reaction mixture was allowed to warm up to 0 °C over a period of 20 min and was further stirred for 10 min at this temperature. Chloroacetonitrile (12 μl; 0.19 mmol) and nBu$_4$NI (0.0584 g; 0.158 mmol) were added and the solution was allowed to warm to

room temperature overnight. The reaction was quenched by NH_4Cl (satd. aq., 40 mL) and extracted with EtOAc (4 × 40 mL). The combined organic phases were washed with brine (20 mL), dried over MgSO4, and the solvent was removed under reduced pressure. Flash column chromatography of the residue (hexanes/EtOAc 3:2) afforded tosylamide as white crystalline solid (0.044 mg; 44%).

R_f 0.60 (hexanes/EtOAc 2:1); mp 86 °C (EtOAc/hexanes); $[\alpha]_D^{20}$ −126.3 (*c* = 1.35, $CHCl_3$); IR (KBr, cm^{-1}) ν 3438, 3291, 3067, 2988, 2937, 2593, 1729, 1601, 1452, 1375, 1353, 1336, 1316, 1274, 1248, 1221, 1248, 1221, 1160, 1096, 1070, 1040, 926, 890, 853, 815, 710, 666, 545, 564; ^1H NMR (600 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.5 Hz, 2H), 7.93 (m, 4H), 7.60 (m, 1H), 7.52 (m, 1H), 7.47 (m, 2H), 7.36 (m, 4H), 5.98 (t, *J* = 3.0 Hz, 1H), 5.69 (m, 1H), 4.92 (br s, 1H), 4.43 (m, 1H), 4.38 (m, 1H), 4.33 (m, 1H), 4.00 (br s, 2H), 2.44 (s, 3H), 2.11 (s, 1H), 1.68 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.0, 164.8, 144.7, 136.2, 133.7, 133.3, 129.97, 129.91, 129.88, 129.3, 129.0, 128.7, 128.4, 128.1, 114.7, 111.0, 75.1, 74.7, 74.0, 71.1, 68.2, 33.5, 28.3, 26.0, 21.6; MS (+EI) *m/z* (%) 614 (1), 613 (3), 246 (13), 171 (12), 155 (5), 105 (100); HRMS (+EI) calcd. for $C_{33}H_{29}N_2O_8S$: 613.16446; found: 613.16387.

(3a*S*,3b*R*,9b*R*,10*S*,11*S*,11a*S*)-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-7,8-bis(trimethylsilyl)-3a,3b,4,5,9b,10,11,11a-octahydro[1,3]benzodioxolo[5,4-*f*]-1,7-naphthyridine-10,11-diyl dibenzoate (472)

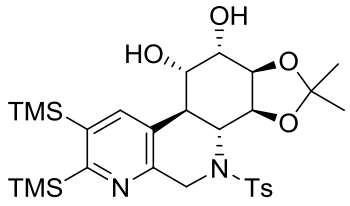


To a solution of $CpCo(CO)_2$ (1 μL; 7.5 μmol) in BTMSA (**269**) (1.5 mL; 0.012 mmol) solution of α,ω-cyanoalkyne **471** (44 mg; 0.07 mmol), $CpCo(CO)_2$ (1 μL; 7.5 μmol) dissolved in *m*-xylene (2 mL), and **269** (1 mL; 0.008 mmol) was added dropwise under irradiation with visible light at 140 °C over period 2 min. The reaction mixture was heated under inert atmosphere for further 24 h. BTMSA and xylene were removed under high vacuum and the residue was purified by column chromatography (gradient hexanes/EtOAc, 9:1 - 6:1). The product **471** was isolated as slightly yellow crystalline foam (26.0 mg, 46.5%).

R_f 0.34 (hexanes/EtOAc 4:1); mp 110 °C (hexane/EtOAc); $[\alpha]_D^{20}$ −25.5 (*c* = 1.45, $CHCl_3$); IR (KBr, cm^{-1}) ν 3435, 3291, 3066, 2983, 2957, 2921, 2851, 1725, 1601, 1528, 1493, 1452, 1406, 1383, 1361, 1346, 1317, 1275, 1250, 1223, 1167, 1107, 1096, 1065, 1028, 912, 842, 755, 710, 666, 555, 541; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.9 Hz, 2H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 8.3 Hz, 2H), 7.54 (m, 2H), 7.37 (m, 5H), 7.05 (d, *J* = 7.9 Hz, 2H), 5.83 (dd, *J*=8.3, 4.2 Hz, 1H), 5.70 (dd, *J*=6.8, 4.5 Hz, 1H), 4.78 (d, J=17.4Hz, 1H), 4.65 (m, 2H), 3.81 (dd, *J*=12.5, 9.4 Hz, 1H), 3.48 (dd, *J*=12.5, 8.3 Hz, 1H), 2.31 (s, 3H), 1.54 (s, 3H), 1.38 (s, 3H), 0.31 (s, 9H), 0.03 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.4, 165.23, 165.18, 151.8, 142.9, 138.9, 137.6, 133.5, 133.4, 129.83, 129.80, 129.3, 129.1, 128.5, 128.4, 127.5, 126.3, 110.6, 75.1, 74.4, 71.1, 69.8, 60.8, 52.8,

38.5, 29.7, 27.9, 25.5, 21.5, 0.98, 0.86; MS (FAB) *m/z* (%) 800 (2), 294 (2), 105 (100), 91 (11); HRMS (+FAB) calcd for $C_{42}H_{51}N_2O_8SSi_2^+$: 799.2905; found: 799.2971.

(3aS,3bR,9bR,10S,11S,11aR)-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-7,8-bis(trimethylsilyl)-3a,3b,4,5,9b,10,11,11a-octahydro[1,3]benzodioxolo[5,4-f]-1,7-naphthyridine-10,11-diol (519)

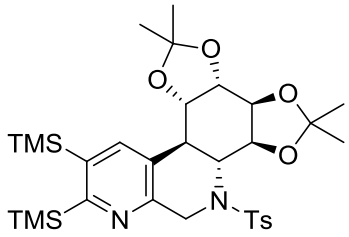


To a solution of **472** (42 mg, 0.052 mmol) in the mixture of CH_2Cl_2:MeOH (4 mL; 1:1) K_2CO_3 (22 mg; 0.16 mmol) was added, and reaction mixture was stirred until disappearance of starting material (TLC). Then the reaction mixture was filtered, evaporated and the residue was purified by column chromatography (gradient hexanes:EtOAc, 2:1 to 1:1). The product was isolated as a transparent foam (19.2 mg, 62%).

R_f 0.2 (hexanes/EtOAc 2:1); $[\alpha]_D^{21}$ − 26.5 (C = 1.0, $CHCl_3$); IR (KBr, cm^{-1}) ν 3467, 2983, 2954, 2926, 2902, 2856, 1446, 1405, 1384, 1356, 1248, 1219, 1161, 1090, 1070, 1050, 858, 841, 756, 715; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70, 7.48 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.1 Hz, 2H), 5.03 (dd, *J* = 8.7, 6.8 Hz, 1H), 4.68 (s, 2H), 4.41-4.36 (m, 1H), 4.13 (dd, *J* = 7.9, 5.3 Hz, 1H), 3.95 (dd, *J* = 7.9, 4.9 Hz, 1H), 3.67-3.60 (m, 1H), 2.94 (s, 1H), 2.85-2.78 (m, 1H), 2.70 (s, 1H), 2.30 (s, 3H), 1.55 (s, 3H), 1.39 (s, 3H), 0.37 (s, 9H), 0.33 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 152.0, 142.6, 139.7, 138.8, 137.3,

128.9, 127.54, 127.50, 110.2, 75.9, 75.7, 70.3, 70.0, 58.8, 53.4, 51.6, 40.0, 27.9, 27.9, 25.2, 21.5, 1.2, 1.1; MS (+EI) m/z (%) 590 (2), 575 (13), 437 (13), 435 (100); HRMS (+EI) calcd for $C_{28}H_{42}N_2O_6SSi^{2+}$: 590.2302; Found: 590.2299.

(3aS,3bS,6aS,6bR,12bR,12cS)-2,2,5,5-tetramethyl-7-[(4-methylphenyl)sulfonyl]-10,11-bis(trimethylsilyl)-3a,3b,6a,6b,7,8,12b,12c-octahydrobis[1,3]dioxolo[3,4:5,6]benzo[1,2-f]-1,7-naphthyridine (520)

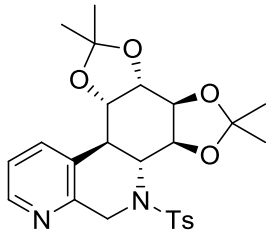


To a solution of diol **519** (0.040 g; 0.068 mmol) in 2,2-dimethoxypropane (1.0 mL; 8.16 mmol) was added *p*-toluenesulfonic acid monohydrate (14 mg, 0.075 mmol). The reaction mixture was stirred until total consumption of starting material (TLC). The reaction mixture was quenched by addition of saturated solution of $NaHCO_3$ (5 ml), extracted by EtOAc (3 × 10 ml). The combined organic phases were dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography (hexanes/EtOAc 2:1)and isolated as colourless oil (0.020 g, 47%).

R_f 0.8 (hexanes:EtOAc 2:1); $[\alpha]^{21}_D$ − 0.4 (*c* = 1.0, $CHCl_3$); IR (KBr, cm^{-1}) ν 2991, 2979, 2936, 2887, 2857, 2852, 1384, 1357, 1244, 1219, 1166, 1106, 1070, 1050, 926, 880, 841, 815, 754; ^1H NMR (300 MHz, $CDCl_3$) δ 7.71, 7.45 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 8.1 Hz, 2H), 4.84 (dd, *J* =8.7, 6.8 Hz, 1H), 4.79-4.73 (m, 1H), 4.56-4.50 (m, 1H), 4.45-4.41 (m, 1H), 4.38-4.33 (m, 2H), 3.53 (dd, *J* = 12.1, 8.7 Hz, 1H), 3.95 (dd, *J* = 11.7, 8.7 Hz,

1H), 2.30 (s, 3H), 1.60 (s, 3H), 1.50 (s, 3H), 1.42 (s, 3H), 1.39 (s, 3H), 0.36 (s, 9H), 0.33 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 152.4, 142.5, 140.0, 138.5, 136.9, 128.9, 127.6, 127.0, 110.2, 110.1, 77.6, 76.36, 76.2, 58.3, 50.3, 39.2, 27.70, 27.66, 25.19, 25.14, 21.5, 1.15, 1.05; MS (+EI) m/z (%) 630 (1), 615 (12), 477 (15),475 (100); HRMS (+EI) calcd for $C_{31}H_{46}N_2O_8SSi^{2+}$: 630.2615; found: 630.2609.

(3aS,3bS,6aS,6bR,12bR,12cS)-2,2,5,5-tetramethyl-7-[(4-methylphenyl)sulfonyl]-3a,3b,6a,6b,7,8,12b,12c-octahydrobis[1,3]dioxolo[3,4:5,6]benzo[1,2-f]-1,7-naphthyridine (521)

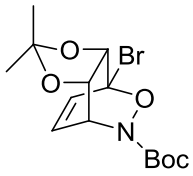


To a solution of acetonide **520** (0.011 g; 0.017 mmol) in THF (2.0 mL) was added solution TBAF in THF (1 M, 0.05 mL, 0.05 mmol). The reaction mixture was stirred until total consumption of starting material (TLC). The reaction mixture was evaporated and the residue was purified by column chromatography (gradient hexanes/EtOAc 2:1 → 1:1) and isolated as colourless oil (0.006 g, 70%).

R_f 0.6 (hexanes:EtOAc 1:2); $[\alpha]^{21}_D$ + 13.0 (*c* = 0.3, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) ν 2998, 2927, 1456, 1384, 1308, 1272, 1259, 1217, 1164, 1070, 924, 881, 817, 712, 696, 669; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.30 (d, *J* = 4.8 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 2H), 7.13 (dd, *J* = 7.6, 4.8 Hz, 1H), 7.11 (d, *J* = 8.4 Hz, 2H), 4.70 (dd, *J* = 9.6, 6.6 Hz, 1H), 4.48-4.42 (m, 2H), 4.38-4.36 (m, 1H), 3.71 (dd, *J* = 12.0, 9.0 Hz, 1H), 2.67 (t, *J*

= 10.8, 1H), 2.36 (s, 3H), 1.61 (s, 3H), 1.50 (s, 3H), 1.45 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) 154.9, 147.1, 143.0, 137.0, 134.1, 130.2, 129.2, 129.0, 127.7, 127.5, 122.8, 110.2, 77.6, 76.43, 76.2, 57.6, 49.3, 39.7, 31.9, 30.91, 29.7, 29.36, 27.7, 27.6, 25.22, 25.19, 21.4;MS (+EI) m/z (%) 486 (6), 279 (10), 167 (25), 150 (11), 149 (79), 123 (12), 43 (100); HRMS (+EI) calcd for $C_{25}H_{30}N_2O_8S^+$: 486.1825; found: 486.1821.

tert-Butyl (3a*S*,4*R*,7*R*,7a*S*)-4-bromo-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino)-1,3-benzodioxole-8-carboxylate (526)

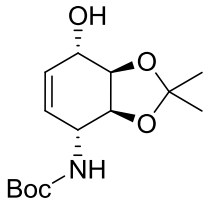


To a solution of diol **4** (3.8 g, 19.8 mmol) in 2,2-dimethoxypropane (10 mL) was added a catalytic amount of *p*-toluenesulfonic acid. After complete consumption of starting material (*vide* TLC), the solution was cooled to 0 °C before water (6 mL) was added. On a preparative scale, the intermediate acetonide **525** was not isolated. $NaIO_4$ (3.83 g, 17.9 mmol) was added to the reaction vessel before *tert*-butyl hydroxycarbamate (2.91 g, 21.8 mmol) in 40 mL of methanol was added dropwise. To ensure complete dissolution 40 mL of CH_2Cl_2 were added. After addition, the solution was allowed to warm to room temperature and stirred for 16 h. Upon completion of the reaction (TLC analysis), an excess of saturated aqueous sodium bisulfite was added carefully until a light straw color was obtained. The mixture was extracted with Et_2O (3 × 100 mL), the organic phase was washed with brine (2 × 15 mL) and dried over Na_2SO_4, and the solvent was removed in

vacuo. The oxazine **526** was isolated by flash column chromatography (hexanes/EtOAc 4:1) as a colourless solid affording (5.31 g, 74%).

R_f 0.6 (hexanes/EtOAc 3:1); mp 155 °C (EtOH), $[\alpha]^{20}_D$ + 25.3 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) ν 3072, 2986, 2936, 1752, 1608, 1472, 1456, 1383, 1294, 1273, 1250, 1210, 1155, 1114, 1071, 1035, 1009, 987, 952, 895, 870, 842, 796, 773, 728, 709, 616, 516, 456; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.45 (d, *J* = 8.1 Hz, 1H), 6.35 (dd, *J* = 8.4, 5.7 Hz, 1H), 4.94-4.92 (m, 1H), 4.56 (s, 2H), 1.42 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.8, 133.8, 131.3, 111.3, 87.4, 83.2, 81.4, 74.2, 53.3, 28.0, 25.6, 25.4; MS (+EI) *m/z* (%) 348 (2), 173 (10), 124 (22), 100 (11), 96 (11), 94 (12), 85 (14), 83(15) 82(17), 57 (100) ; HRMS (+EI) calcd for $C_{14}H_{20}BrNO_5^+$: 361.0525; found: 361.0528; Anal. calcd for $C_{14}H_{20}BrNO_5$: C, 46.42; H, 5.57. Found C, 46.51; H, 5.55.

***tert*-Butyl [(3a*S*,4*R*,7*S*,7a*R*)-7-hydroxy-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl]carbamate (527)**

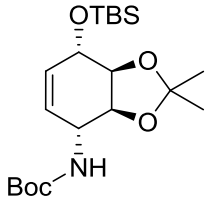


To a solution of **526** (1.02 g, 2.81 mmol) in THF:H_2O (70:7 mL) was added an aluminum amalgam prepared by dipping aluminum foil (2.5 g, 92.9 mmol) sequentially to NaOH (1 M), distilled water, $HgCl_2$ (0.5% solution), distilled water, THF. After overnight stirring, the reaction mixture was filtered through diatomaceous earth, washed by methanol (3 × 50 mL). Filtrate was evaporated, redissolved in toluene and evaporated again. The

reaction product was isolated by flash column chromatography (hexanes/EtOAc 1:1) affording **527** as colourless viscous oil (0.714 g, 89%).

R_f 0.3 (hexanes/EtOAc 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.91 (ddd, J = 9.8, 2.2, 2.2 Hz, 1H), 5.80 (ddd, J = 9.8, 2.9, 1.0 Hz, 1H), 5.07 (b, 1H), 4.27-16 (m, 3H),4.02 (m, 1H), 2.63(b, 1H), 1.45 (s, 12H), 1.35 (s, 3H).^{1}H NMR matched with lit. value.[235]

***tert*-Butyl ((3a*S*,4*R*,7*S*,7a*S*)-7-{[*tert*-butyl(dimethyl)silyl]oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)carbamate (528)**

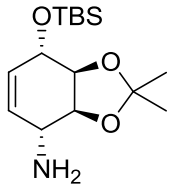


To a solution of **527** (0.372 g, 1.3 mmol) in CH_2Cl_2 (20 mL) was added imidazole (0.133g, 1.96 mmol) followed by TBSCl (0.215g, 1.43 mmol). After overnight stirring, the reaction mixture quenched by water (10 mL), extracted by CH_2Cl_2 (3 × 10 mL), dried and evaporated. The reaction product was isolated by flash column chromatography (hexanes/EtOAc 8:1) affording **528** as a colourless liquid (0.425 g, 81%).

R_f 0.9 (hexanes/EtOAc 3:1); $[\alpha]^{20}_D$ − 0.8 (c = 1.0, $CHCl_3$), IR ($CHCl_3$, cm^{-1}) ν 3394, 3055, 2991, 2973, 2962, 2933, 2908, 2860, 1710, 1510, 1463, 1382, 1369, 1325, 1294, 1252, 1211, 1167, 1097, 1059, 1038, 978, 966, 937, 914;^{1}H NMR (300 MHz, $CDCl_3$) δ 5.98-5.93 (m, , 1H), 5.35 (d, J = 8.7 Hz, 1H), 4.28-4.22 (m, 3H), 4.15-4.13 (m, 1H), 1.39 (s, 9H), 1.33 (s, 3H), 1.27 (s, 3H), 0.88 (s, 9H) 0.10 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.2, 131.8, 131.2, 108.1, 79.2, 78.8, 67.1, 47.6, 28.3, 26.4, 25.7, 24.4, 17.9, −4.80, −4.99; MS (+EI) *m/z* (%) 299 (16), 286 (37), 256 (23), 244 (13), 243 (75),

238 (16), 228 (54), 225 (17), 215 (24), 212 (17), 210 (38), 199 (28), 196 (11), 184 (14), 182 (12), 168 (24), 167 (100); HRMS (+EI) calcd for $C_{20}H_{37}NO_5Si^+$: 399.2441; found: 399.2435; Anal. calcd for $C_{20}H_{37}NO_5Si$: C, 60.11; H, 9.33. Found C, 60.36; H, 9.23.

((3a*S*,4*R*,7*S*,7a*S*)-7-{[*tert*-Butyl(dimethyl)silyl]oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)amine (473)

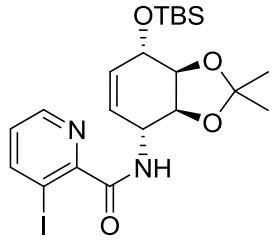


Trifluoroacetic acid (7 mL) was added dropwise to a solution of **528** (6.01 g, 15.04 mmol) in CH_2Cl_2 (150 mL) at 0 °C. After 15 min reaction has been neutralised by addition of concentrated ammonia (50 mL). Organic layer was extracted by CH_2Cl_2 3 × 50 mL, washed with brine, dried over Na_2SO_4 and evaporated. The free amine **473**, 3.86 g (86%), was obtained as colourless oil and of sufficient purity to use in the next step. Analytical sample of **473** was purified by flash column chromatography (CH_2Cl_2/CH_3OH 10:1).

R_f 0.8 (CH_2Cl_2/ CH_3OH 10:1); $[\alpha]^{20}_D$ + 15.5 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) ν 3424, 3371, 3323, 3048, 2985, 2955, 2933, 2898, 2858, 1677, 1635, 1600, 1467, 1383, 1255, 1212, 1163, 1117, 1060, 887, 839, 780; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.73 (dt, *J* = 9.8, 2.3 Hz, 1H), 5.65 (dt, *J* = 9.8, 2.3 Hz, 1H), 4.19-4.16 (m, 1H), 4.11 (dd, *J* = 7.8, 4.8 Hz, 1H), 3.89 (dd, *J* = 7.5, 6.3 Hz, 1H), 3.28 (dd, *J* = 5.7, 2.1 Hz, 1H), 1.66 (s, 2H), 1.44 (s, 3H), 1.34 (s, 3H), 0.91 (s, 9H) 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ

132.3, 131.4, 108.7, 81.0, 80.6, 71.4, 52.0, 27.1, 25.8, 24.7, 18.3, −4.6, −4.9; MS (+EI) *m/z* (%) 242 (10), 215 (12), 212 (33), 200 (17), 199 (100); HRMS (+EI) calcd for $C_{15}H_{29}NO_3Si^+$: 299.1917; found: 299.1903; Anal. calcd for $C_{15}H_{29}NO_3Si$: C, 60.16; H, 9.76. Found C, 60.31; H, 9.57.

_N_-((3a*S*,4*R*,7*S*,7a*S*)-7-{[*tert*-Butyl(dimethyl)silyl]oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)-3-iodopyridine-2-carboxamide (529)

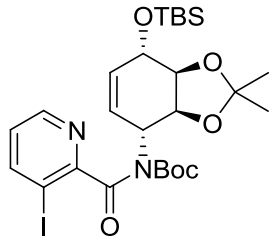


To a solution of lithium salt of 3-iodopicolinic acid[227] (3.23 g, 12.68 mmol) in DMF (60 mL) was added HBTU (3.93 g, 10.35 mmol), stirred for 5 min at 0 °C, followed by solution of **473** (3.67 g, 9.41 mmol) and diisopropylethylamine (2.46 mL, 14.12 mmol) in CH_2Cl_2 (100 ml). After 1 h stirring, the reaction mixture quenched by water (50 mL), extracted by CH_2Cl_2 (3 × 100 mL), dried over Na_2SO_4 and evaporated. The reaction product was isolated by flash column chromatography (hexanes/EtOAc 4:1) affording **529** as a colourless oil (3.27 g, 66%).

R_f 0.85 (hexanes/EtOAc 2:1); $[\alpha]^{20}_D$ − 38.3 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) ν 3387, 2986, 2953, 2930, 2901, 2856, 1678, 1507, 1462, 1456, 1383, 1254, 1212, 1164, 1120, 1061, 1014; ^1H NMR (600 MHz, $CDCl_3$) δ 8.50 (dd, *J* = 4.5, 1.5 Hz, 1H), 8.31 (dd, *J* = 8.2, 1.5 Hz, 1H), 8.21 (d, *J* = 9.1 Hz, 1H), 7.08 (dd, *J* = 7.9, 4.5 Hz, 1H), 6.06 (dd, *J* = 9.8, 4.2 Hz, 1H), 5.98 (dd, *J* = 9.8, 4.9 Hz, 1H),4.78-4.76 (m, 1H), 4.41 (dd, *J* = 7.2, 3.8

Hz, 1H), 4.30 (dd, J = 7.2, 3.4 Hz, 1H), 4.27 (dd, J = 4.2, 3.4 Hz, 1H) 1.42 (s, 3H), 1.33 (s, 3H), 0.9 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.7, 150.4, 148.8, 147.2, 132.9, 129.5, 126.2, 108.5, 89.6, 76.7, 68.2, 47.7, 26.7, 25.8, 25.7, 24.6, 18.2, − 4.7, − 4.8; MS (+EI) *m/z* (%) 515 (6), 436 (26), 430 (31), 415 (29), 305 (14), 298 (24), 289 (12), 282 (18) 249 (30), 232 (75), 215 (15), 205 (12), 204 (53), 185 (13), 168 (15), 167 (83), 157 (20), 150 (10), 149 (35), 128 (10), 106 (16), 86 (20), 85 (10), 84 (32), 79 (19), 78 (33), 77 (26), 76 (13), 75 (100); HRMS (+EI) calcd for $C_{20}H_{28}IN_2O_4Si^+$: 515.0863; found: 515.0868; Anal. calcd for $C_{21}H_{31}IN_2O_4Si$: C, 47.55; H, 5.89. Found C, 47.84; H, 5.90.

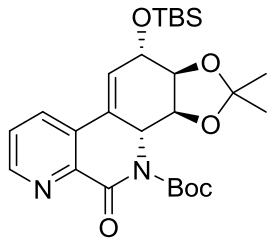
tert-Butyl ((3a*S*,4*R*,7*S*,7a*S*)-7-{[*tert*-butyl(dimethyl)silyl]oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)[(3-iodopyridin-2-yl)carbonyl]carbamate (475)



To a solution of amide **529** (0.090 g, 0.17 mmol) in acetonitrile (2 mL) was added di-*tert*-butyldicarbonate (0.083 g, 0.38 mmol), and DMAP (0.047 g, 0.38 mmol). After 2 h stirring, the reaction mixture quenched by water (5 mL), extracted by CH_2Cl_2 (3 × 10 mL), dried and evaporated. The reaction product was isolated by flash column chromatography (hexanes/EtOAc 4:1) affording **475** as a colourless oil (0.098 g, 92%). R_f 0.9 (hexanes/EtOAc 2:1); $[\alpha]^{20}_D$ − 11.0 (c = 1.0, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) ν 3449, 3050, 2983, 2955, 2934, 2901, 2858, 1743, 1684, 1473, 1461, 1430, 1387, 1353, 1343,

1254, 1218, 1154, 1112, 1062, 1010, 888, 839, 777; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (dd, *J* = 4.8, 1.2 Hz, 1H), 8.14 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.07 (dd, *J* = 8.1, 4.8 Hz, 1H), 5.71(s, 2H), 5.26(br.s, 1H), 4.67 (m, 1H), 4.24 (m, 1H), 4.16 (m, 1H), 1.51 (s, 3H), 1.37 (s, 3H), 1.22 (s, 9H), 0.94 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2, 158.7, 151.6, 147.6, 146.6, 131.1, 126.4, 124.8, 108.6, 89.0, 84.4, 80.1, 75.1, 71.2, 27.8, 27.5, 25.9, 25.7, 18.2, − 4.5, − 4.9; MS (+EI) *m/z* (%) 530 (10), 473 (32), 459 (16), 430 (19), 415 (40), 305 (11), 298 (19), 282 (14) 260 (38), 249 (44), 232 (63), 204 (37), 168 (15), 167 (68), 157 (12), 57 (100); HRMS (+EI) calcd for $C_{26}H_{39}IN_2O_6Si^{+}$: 630.1622; found: 630.1615; Anal. calcd for $C_{26}H_{39}IN_2O_6Si$: C, 49.52; H, 6.23. Found C, 49.66; H, 6.24.

tert-Butyl (3a*S*,3b*R*,11*S*,11a*S*)-11-{[_tert_-butyl(dimethyl)silyl]oxy}-2,2-dimethyl-5-oxo-3a,5,11,11a-tetrahydro[1,3]benzodioxolo[5,4-*f*]-1,7-naphthyridine-4(3b*H*)-carboxylate (530)

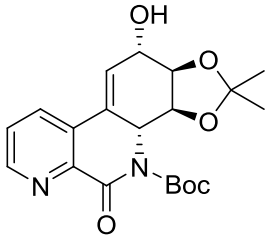


To a solution of **475** (1.30 g, 2.06 mmol) in toluene (45 mL) was added $Pd(OAc)_2$ (0.093 g, 0.41 mmol) and Ag_3PO_4 (0.520 g, 1.24 mmol). The reaction mixture was degassed by passing argon for 5 min followed by addition of 1,2-bis(diphenylphosphino)ethane (0.164 g, 0.41 mmol). After addition of dppe the reaction mixture was refluxed for 18 h. Product was isolated by flash column chromatography (hexanes/EtOAc 2:1 → 1:1) affording 0.32

g (31%, 46% based on recovered starting material) of **530** as a colourless oil and 0.440 g of starting material.

R_f 0.2 (hexanes/EtOAc 1:1); $[\alpha]^{20}_D$ + 18.1 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) ν 3450, 3074, 2984, 2955, 2933, 2903, 2858, 1756, 1691, 1634, 1469, 1382, 1254, 1215, 1155, 1125, 1068, 1014, 964, 920, 843, 782; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (dd, *J* = 4.2, 0.9 Hz, 1H), 7.99 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.44 (dd, *J* = 8.4, 4.5 Hz, 1H), 6.51-6.49 (m, 1H), 4.90 (dt, *J* = 7.8, 2.4 Hz 1H), 4.46-4.44 (m, 1H), 4. (dd, *J* = 8.1, 2.7 Hz, 1H), 1.61 (s, 9H), 1.50 (s, 3H), 1.33 (s, 3H), 0.96 (s, 9H), 0.18 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 153.2, 150.8, 141.5, 131.2, 130.3, 128.4, 126.9, 125.8, 111.7, 84.3, 79.9, 79.7, 73.6, 57.9, 27.6, 26.9, 25.8, 25.2, 18.2, - 4.5, - 5.0; MS (+FAB) *m/z* (%) 503 (5), 419 (12), 404 (77), 303 (12), 287 (10), 185 (13), 185 (13), 75 (10), 73 (100); HRMS (+FAB) calcd for $C_{26}H_{39}N_2O_6Si^+$: 503.2577; found: 503.2538.

tert-Butyl (3a*S*,3b*R*,11*S*,11a*R*)-11-hydroxy-2,2-dimethyl-5-oxo-3a,5,11,11a-tetrahydro[1,3]benzodioxolo[5,4-*f*]-1,7-naphthyridine-4(3b*H*)-carboxylate (531)

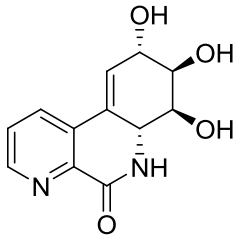


Naphthridone **530** (0.121 g, 0.024 mmol) was dissolved in THF (2.5 mL) and cooled to 0 °C. A solution of TBAF in THF (1 M, 0.25 mL, 0.25 mmol) was added dropwise over 1 min. The reaction mixture was stirred until total consumption of starting material was

observed (TLC). The reaction mixture was quenched with solution of NH_4Cl (2 ml, sat. aq. solution) and extracted with EtOAc (6 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated. Compound **531** was isolated by column chromatography (CH_2Cl_2/methanol 100:1) as white crystalline solid (0.072 mg, 77%).

R_f 0.8 (CH_2Cl_2/methanol 10:1); mp > 200 °C (CH_2Cl_2); $[\alpha]^{20}_D$ − 4.1 (*c* = 0.2, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) ν 3431, 2983, 2935, 2877, 1765, 1684, 1627, 1473, 1384, 1248, 1217, 1153, 1115, 1070; ^1H NMR (300 MHz, $CDCl_3$) δ 8.69 (d, *J* = 3.9 Hz, 1H), 8.00 (d, *J* = 8.1 Hz, 1H), 7.42 (dd, *J* = 8.1, 4.5 Hz, 1H), 6.72 (t, *J* = 2.7 Hz, 1H), 4.87-4.84 (m, 1H), 4.53-4.52 (m, 1H), 4.38 (br s, 1H), 4.25-4.14 (m, 1H), 1.56 (s, 9H), 1.49 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.6, 152.9, 150.6, 141.1, 130.7, 128.7, 127.2, 125.4, 111.9, 84.5, 79.7, 79.2, 72.5, 58.2, 27.4, 26.9, 25.1; MS (+FAB) *m/z* (%) 389 (5), 290 (12), 289 (60), 243 (14), 242 (74), 186 (44), 185 (13), 184 (15), 142 (22), 81 (12), 77 (10), 73 (18), 69 (24), 67(12), 57 (100); HRMS (+FAB) calcd for $C_{20}H_{25}N_2O_6^+$:389.1713; found: 389.1694.

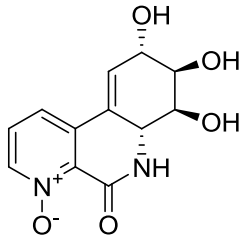
(6a*R*,7*S*,8*R*,9*S*)-7,8,9-Trihydroxy-5-oxo-5,6,6a,7,8,9-hexahydrobenzo[*f*]-1,7-naphthyridin-4-ium chloride (6)



Compound **531** (0.072 g, 0.185 mmol) was taken up in methanol (2.5 mL) and HCl (conc., 0.25 mL) was added dropwise. The reaction mixture was stirred at r.t. until total consumption of starting material was observed (TLC). The reaction mixture was evaporated and dried *in vacuo* and off-white crystals of hydrochloride of **6** were obtained (53 mg, quantitative). Upon chromatography on silica gel (deactivated with 10% w/w water, eluent CH_2Cl_2/MeOH/NH_3=90:10:1) free base of **6** was isolated (0.040 g, 86 %).

R_f 0.35 (CH_2Cl_2/methanol/ammonia 80:35:4); mp > 200 °C (methanol); $[\alpha]^{20}_D$ − 10 (*c* = 0.5, H_2O); IR ($CHCl_3$, cm^{-1}) ν 3130, 3037, 2850, 2877, 1655, 1401, 1322, 1087, 1029; for **6*HCl**: ^1H NMR (600 MHz, D_2O) δ 8.74-8.72 (m, 2H), 8.07 (s, 1H), 6.55 (t, *J* = 3.0 Hz, 1H), 4.51 (d, *J* = 8.4 Hz, 1H), 4.34 (t, *J* = 2.4 Hz, 1H), 3.98 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.96 (s, 1H); ^{13}C NMR (150 MHz, D_2O) δ 158.8, 142.8, 141.8, 136.4, 134.6, 130.1, 129.3, 126.9, 72.2, 68.6, 68.4, 52.1; MS (+FAB) *m/z* (%) 389 (5), 290 (12), 289 (60), 243 (14), 242 (74), 186 (44), 185 (13), 184 (15), 142 (22), 81 (12), 77 (10), 73 (18), 69 (24), 67(12), 57 (100); HRMS (+FAB) calcd for $C_{12}H_{13}N_2O_4^+$:249.0875; found: 249.0847.

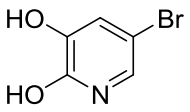
(6a*R*,7*S*,8*R*,9*S*)-7,8,9-trihydroxy-6a,7,8,9-tetrahydrobenzo[*f*]-1,7-naphthyridin-5(6*H*)-one 4-oxide (476)



Triol **6** (0.052 g, 0.21mmol) was dissolved in a mixture of methanol (2.5 mL) and $CHCl_3$ (7.5 mL) and 100% *m*-chloroperbenzoic acid (103 mg, 0.6 mmol) was added in one portion. The reaction mixture was stirred at r.t. until consumption of starting material (TLC). After that period the reaction mixture was evaporated and subjected to a column chromatography on silica gel (deactivated with 10% w/w water, eluent CH_2Cl_2/MeOH/NH_3=80:35:4) to afford off-white crystals of **476** (0.014 g, 25%).

R_f 0.05 (CH_2Cl_2/methanol/ammonia 80:35:4); mp > 200 °C (methanol); $[\alpha]^{20}_D$ + 733 (*c* = 0.1, methanol); IR ($CHCl_3$, cm^{-1}) ν 3483, 2982, 2930, 2877, 1740, 1670, 1627, 1473, 1400, 1265, 1212, 1155, 1112, 1074; ^{1}H NMR (600 MHz, CD_3OD) δ 8.35 (d, *J* = 6.6 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 6.52 (dd, *J* = 4.2, 1.8 Hz, 1H), 6.52 (d, *J* = 2.5 Hz, 1H), 4.36 (d, *J* = 8.4 Hz, 1H), 4.30 (t, *J* = 3.6 Hz 1H), 3.`97 (dd, *J* = 8.4, 2.4 Hz, 1H), 3.94 (br s, 1H); ^{13}C NMR (600 MHz, CD_3OD) δ 158.4, 141.5, 137.8, 135.4, 129.3, 128.0, 127.5, 123.1, 72.7, 69.3, 69.1, 52.2; MS (+FAB) *m/z* (%) 265 (34), 207 (10), 192 (14), 177 (16), 176 (34), 171 (19), 165 (10), 163 (16), 151 (10), 149 (15), 136 (41), 121 (12), 113 (10), 109(20), 107 (25), 105 (21), 55 (100); HRMS (+FAB) calcd for $C_{12}H_{13}N_2O_5^+$:265.0825; found: 265.0826.

5-bromo pyridine-2,3-diol (534)

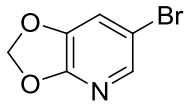


Freshly distilled furfural (51 mL, 0.615 mol) was mixed with 700 g of ice and bromine (32 mL, 0.615 mol) was added dropwise to a vigorous stirred reaction mixture while maintaining temperature 0 °C. After 30 min of stirring HCl (conc., 30 mL) was added in

one portion and stirred for an additional 30 min. Bromine (32 mL, 0.615 mol) was added dropwise while maintaning temperature −5 °C in a period of 1 h. The reaction mixture was then filtered and sulfamic acid (60 g, 0.62 mol) was added to a filtrate and vigorously stirred for 1.5 h at 50 °C. The reaction mixture was cooled to 10 °C and filtered. Precipitate was dried for 18 h at 50 °C, then refluxed in glacial acetic acid with charcoal, and recrystallized from acetic acid. Crystals were washed with distilled water till neutral reaction and dried *in vacuo* to obtain **534** as grey crystals (76 g, 65%).

mp 248-250 °C (AcOH), [lit.[202] 249 °C (AcOH)].

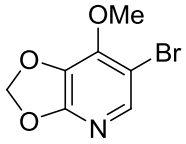
6-bromo[1,3]dioxolo[4,5-*b*]pyridine (533)



To a solution of 5-bromo pyridine-2,3-diol **534** (0.700 g, 3.7 mmol) in 30 ml of DMF was added K_2CO_3(0.50 g, 7.4 mmol), CH_2Br_2 (0.75 ml, 7.4 mmol) and CuO (0.120 g, 0.74 mmol). Mixture was heated to 95 °C for 6 h, then cooled down, filtered, diluted with H_2O (300 mL) and extracted with EtOAc (5 × 10 ml). Organic phases were dried over Na_2SO_4 and subjected to column chromatography (Hex/EtOAc 4:1). Product was isolated as white needles (0.135 g, 18%).

mp 65-67°C (Hexanes-EtOAc), [Lit.[202] 69-71°C (EtOH)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, J = 1.9 Hz, 1 H), 7.12 (d, J = 1.9 Hz, 1 H), 6.10 (s, 2H).

6-bromo-7-methoxy[1,3]dioxolo[4,5-*b*]pyridine (532)

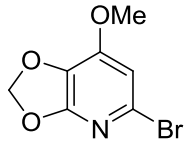


To a solution of 2,2,6,6-tetramethylpiperidine (6.04 mL, 35.5 mmol) in THF (50 mL) at -50°C *n*BuLi (14.30 mL, 30.8 mmol) was added dropwise. Reaction mixture was stirred for 15 min at −30 °C, then cooled down to −80 °C and solution of **533** (4.88 g, 23.7 mmol) in THF (20 mL) was added dropwise, while maintaining temperature −80 °C. Reaction mixture was stirred for an additional 30 min, followed by addition of $B(OMe)_3$ (6.6 mL, 59.1 mmol) in one portion and reaction mixture was warmed up to 0 °C within 30 min. Then reaction mixture was cooled to −50 °C and glacial acetic acid (6.76 mL, 118 mmol) was added, followed by addition of UHP (10 g) and stirred overnight at r.t. Next morning excess of peroxide was quenched by $NaHSO_3$ (sat. aq.), extracted with EtOAc (3×150 mL). Organic layer was dried over Na_2SO_4, evaporated and redissolved in THF:MeOH mixture (10:1, 200 mL) at 0 °C. Ethereal solution of diazomethane was added dropwise until disappearance of starting material was observed (TLC). Reaction mixture was evaporated and subjected to flash column chromatography (silica gel, hexane/EtOAc 4:1) to yield **532** as a white crystalline compound (2.0 g, 36%).

R_f 0.45 (hexanes/EtOAc 4:1); mp 119-121 °C ($CHCl_3$); IR (film, cm^{-1}) ν 3092, 3003, 2957, 2924, 2853, 1786, 1614, 1492, 1472, 1431, 1401, 1277, 1268, 1232, 1202, 1146, 1091, 1033, 982; ^1H NMR (600 MHz, $CDCl_3$) δ 7.74 (s, 0.75 H), 7.62 (s, 0.2 H), 6.05 (s, 2H), 4.21 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.6, 145.7, 141.6, 139.1, 127.3, 105.4, 100.5, 100.4, 59.8; MS (+EI) *m/z* (%) 233 (97), 231 (100), 203 (23), 187 (43), 175

(16), 173 (16), 160 (88), 158 (84), 132 (31), 131 (33), 130 (33), 129 (12); HRMS (+EI) calcd for $C_7H_6BrNO_3$: 232.9512; found: 232.9507.

5-bromo-7-methoxy[1,3]dioxolo[4,5-*b*]pyridine (538)

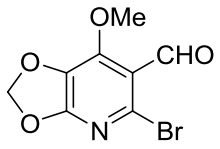


To a solution of 2,2,6,6-tetramethylpiperidine (0.320 mL, 1.86 mmol) in THF (5 mL) at -50°C *n*BuLi (0.770 mL, 1.82 mmol) was added dropwise. Reaction mixture was stirred for 15 min at −30 °C, then cooled down to −80 °C and added dropwise to a solution of **532** (0.206 g, 0.89 mmol) in THF (5 mL) while maintaining temperature −80 °C. Reaction mixture was stirred for 20 min at this temperatute, followed by consequtive quenching with methanol (1 mL) and solution of NH_4Cl (sat. aq., 2 mL). Reaction mixture was extracted with extracted with EtOAc (3 × 30 mL). Organic layer was dried over Na_2SO_4, evaporated subjected to flash column chromatography (silica gel, hexane/EtOAc 4:1) to yield **538** as a white crystalline compound (0.110 g, 54%).

R_f 0.40 (hexanes/EtOAc 4:1); mp 113-115 °C ($CHCl_3$); IR (film, cm^{-1}) ν 3107, 3003, 2950, 2924, 1789, 1632, 1591, 1504, 1476, 1453, 1428, 1417, 1334, 1281, 1191, 1129, 1105, 1030, 958, 921, 913, 870, 830 775; ^1H NMR (300 MHz, $CDCl_3$) δ 6.69 (s, 1H), 6.05 (s, 2H), 3.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.9, 149.9, 141.6, 130.4, 126.9, 109.2, 100.9, 57.2; MS (+EI) *m/z* (%) 233 (100), 231 (98), 203 (13), 201 (13), 160

(78), 158 (73), 132 (18), 130 (19), 94 (12); HRMS (+EI) calcd for $C_7H_6BrNO_3$: 232.9512; found: 232.9516.

5-bromo-7-methoxy[1,3]dioxolo[4,5-*b*]pyridine-6-carbaldehyde (539)

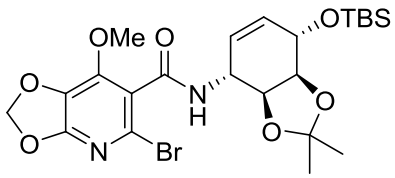


To a solution of 2,2,6,6-tetramethylpiperidine (0.137 mL, 0.81 mmol) in THF (5 mL) at −50°C *n*BuLi (0.324 mL, 0.77 mmol) was added dropwise. Reaction mixture was stirred for 15 min at −30 °C, then cooled down to -80 °C and added dropwise to a solution of **532** (0.170 g, 0.73 mmol) in THF (5 mL) while maintaining temperature −80 °C. The reaction mixture was stirred for 20 min at this temperature, followed by quenching with dry DMF (1 mL), followed by addition of NH_4Cl (sat. aq., 2 mL). The reaction mixture was extracted with EtOAc (3 × 20 mL). Organic layer was dried over Na_2SO_4, evaporated, and subjected to flash column chromatography (silica gel, hexane/EtOAc 4:1 to 2:1) to yield **539** (0.015 g, 8%) as a off-white crystalline compound and **538** as white crystalline compound (0.062 g,36 %).

R_f 0.2 (hexanes/EtOAc 2:1); mp 130-135 °C (CH_2Cl_2); IR (film, cm^{-1}) ν 3110, 3000, 2950, 2825, 2720, 1730, 1630, 1600, 1496, 1466, 1432, 1418, 1411, 1334, 1281, 1191, 1129, 1105, 1040, 960; ^1H NMR (300 MHz, $CDCl_3$) δ 10.22 (s, 1H), 6.13 (s, 2H), 4.23 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.8, 161.1, 150.2, 137.2, 127.0, 118.0, 101.1, 60.4; MS (+EI) *m/z* (%) 261 (97), 259 (100), 244 (16), 243 (12), 229 (14), 201 (10), 188

(39), 186 (40), 150 (49), 133 (13), 132 (24), 131 (13), 130 (23), 122 (12), 94 (16); HRMS (+EI) calcd for $C_8H_6BrNO_4$: 258.9480; found: 258.9470.

5-bromo-*N*-((3a*S*,4*R*,7*S*,7a*S*)-7-{[*tert*-butyl(dimethyl)silyl]oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)-7-methoxy[1,3]dioxolo[4,5-*b*]pyridine-6-carboxamide (541)



A. Synthesis from **532:**

To a solution of 2,2,6,6-tetramethylpiperidine (0.240 mL, 1.40 mmol) in THF (30 mL) at −50°C *n*BuLi (0.640 mL, 1.37 mmol) was added dropwise.The reaction mixture was stirred for 15 min at −30 °C, then cooled down to −85 °C and was added dropwise to a solution of **532** (0.310 g, 1.336 mmol) in THF (20 mL), while maintaining temperature -85°C. The reaction mixture was stirred for 30 min at this temperature, followed by a quick addition to a solid CO_2 and evaporation. Solid residue was redissolved in acetonitrile (50 mL) and HBTU (0.455 g, 1.6 mmol) was added, followed by DIPEA (0.470 mL, 2.67 mmol) and solution of amine **473** (0.480 g, 1.6 mmol) in CH_2Cl_2 (2 mL). Reaction mixture was stirred for five hours, quenched with NH_4Cl (sat. aq., 2 mL) extracted with EtOAc (3 × 10 mL). Combined organic layer was dried over Na_2SO_4, evaporated subjected to flash column chromatography (silica gel, hexane/EtOAc 3:1 to 2:1) to yield **541** as a white crystalline compound (0.224 g, 30% based on **532**).

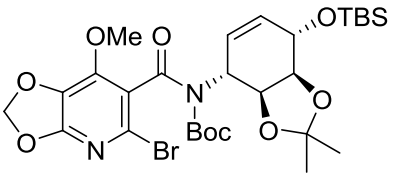
B. Synthesis from **538**:

To a solution of 2,2,6,6-tetramethylpiperidine (0.070 mL, 0.41 mmol) in THF (10 mL) at −50°C *n*BuLi (0.173 mL, 0.41 mmol) was added dropwise. The reaction mixture was stirred for 15 min at −30 °C, then cooled down to −85 °C and solution of **538** (0.091 g, 0.39 mmol) in THF (4 mL) was added dropwise to LTMP, while maintaining temperature -85 °C. The reaction mixture was stirred for 3 min at this temperature, followed by a quick addition to a solid CO_2 and evaporation. Solid residue was redissolved in acetonitrile (5 mL) and HBTU (0.178 g, 0.47 mmol) was added, followed by DIPEA (0.101 g, 0.784 mmol) and solution of amine **473** (0.130g, 0.43 mmol) in CH_2Cl_2 (2 mL). Reaction mixture was stirred for five hours, quenched with NH_4Cl (sat. aq., 2 mL) extracted with EtOAc (3 × 10 mL). Combined organic layer was dried over Na_2SO_4, evaporated subjected to flash column chromatography (silica gel, hexane/EtOAc 3:1 to 2:1) to yield **541** as a white crystalline compound (0.114 g, 52% based on **538**).

R_f 0.6 (hexanes/EtOAc 3:2); mp 153-161°C ($CHCl_3$); $[\alpha]^{20}_D$ − 11.9 (*c* = 1, MeOH); IR (film, cm^{-1}) ν 3641, 3402, 3264, 3054, 2956, 2931, 2858, 2720, 1654, 1624, 1596, 1535, 1502, 1466, 1439, 1413, 1394, 1344, 1324, 1303, 1258, 1212, 1164, 1103, 1061, 999, 841;^{1}H NMR (300 MHz, $CDCl_3$) δ 6.73 (d, *J* = 9.5 Hz, 1H), 6.17 (d, *J* = 2.5 Hz, 2H), 6.02 (s, 2H), 4.88-4.85 (m, 1H), 4.58 – 4.49 (m, 1H), 4.36 (d, *J* = 6.8 Hz, 1H), 4.21 (s, 1H), 4.09 (s, 3H), 1.36 (s, 3H), 1.31 (s, 3H), 0.72 (s, 9H), 0.06 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.3, 159.2, 146.9, 132.7, 130.4, 127.9, 126.3, 122.0, 108.4, 100.6, 78.3, 77.1, 76.4, 66.3, 59.9, 45.9, 26.3, 25.4, 24.4, 17.6, -4.9, -5.1; MS (+EI) *m/z* (%) 501(10), 499 (10), 458 (10), 284 (14), 282 (13), 260 (32), 258 (33), 246 (26), 243

(23), 242 (11), 241 (17), 226 (16), 215 (19), 212 (27), 200 (15), 199 (65), 75 (100); HRMS (+EI) calcd for $C_{22}H_{30}BrN_2O_7Si$: 541.1006; found: 541.1003.

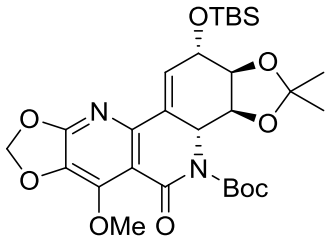
tert-butyl [(5-bromo-7-methoxy[1,3]dioxolo[4,5-*b*]pyridin-6-yl)carbonyl]((3a*S*,4*R*,7*S*,7a*S*)-7-{[*tert*-butyl(dimethyl)silyl]oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)carbamate (478)



To a solution of amide **541** (0.568 g, 1.02 mmol) in acetonitrile (20 mL) was added di-*tert*-butyldicarbonate (0.444 g, 2.04 mmol), and DMAP (0.25 g, 2.07 mmol). The reaction was stirred for 12 h and then quenched with water (5 mL), extracted by CH_2Cl_2 (3 × 20 mL), dried and evaporated. The product was isolated by flash column chromatography (hexanes/EtOAc 4:1) affording **478** as a colorless oil (0.470 g, 70%). R_f 0.8 (hexanes/EtOAc 2:1); $[\alpha]^{17}_D$ – 19.4 (*c* = 1, $CHCl_3$); IR (film, cm^{-1}) ν 3431, 2959, 2932, 2858, 1742, 1680, 1625, 1598, 1466, 1438, 1418, 1396, 1371, 1332, 1261, 1235, 1154, 1103, 1061, 1020; ^{1}H NMR (600 MHz, Acetone-d6) δ 6.25 (s, 1H), 6.20 (s, 1H), 5.71 – 5.63 (m, 2H), 5.26 (s, 1H), 4.61 (dd, *J* = 6.6, 4.8 Hz, 1H), 4.27 – 4.23 (m, 1H), 4.15 (d, *J* = 1.2 Hz, 3H), 4.09 (dd, *J* = 12.6, 6.0 Hz, 1H), 1.48 (d, *J* = 3.0 Hz, 3H), 1.35 (d, *J* = 4.2 Hz, 9H), 1.34 (d, *J* = 4.8 Hz, 3H), 0.96 (s, 9H), 0.19 (s, 3H), 0.18 (s, 3H); ^{13}C NMR (151 MHz, Acetone) δ 165.0, 164.8, 159.2, 159.1, 151.5, 146.3, 145.9, 130.5, 130.4, 127.1, 126.9, 125.7, 124.6, 124.0, 123.9, 108.1, 108.1, 101.3, 84.0, 84.0, 79.8,

79.8, 75.3, 71.3, 71.3, 59.7, 59.7, 27.3, 27.2, 26.9, 25.4, 24.9, 17.8, −5.1, −5.54, HRMS (+EI): $[M-CH_3-tBu-CO_2]^+$ calcd for $C_{22}H_{30}BrN_2O_7Si$: 541.1000; found: 541.1004.

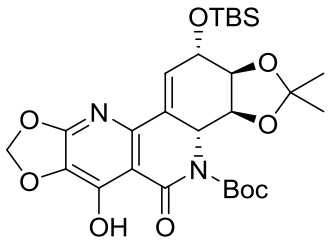
tert-Butyl (3a*S*,3b*R*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-6-methoxy-2,2-dimethyl-5-oxo-3a,5,12,12a-tetrahydro[1,3]benzodioxolo[4,5-*h*][1,3]dioxolo[4,5-*b*]-1,6-naphthyridine-4(3b*H*)-carboxylate (542)



To a solution of **478** (0.30 g, 0.457 mmol) in toluene (35 mL) was added $Pd(OAc)_2$ (0.025 g, 0.113 mmol) and Ag_3PO_4 (0.140 g, 0.33 mmol). The reaction mixture was degassed by passing argon for 5 min followed by addition of 1,2-*bis* (diphenylphosphino)ethane (0.036 g, 0.091 mmol). After addition of dppe the reaction mixture was stirred at 95 °C for 20 h. Product was isolated by flash column chromatography (hexanes/EtOAc 2:1 → 1:1) affording **542** as a white solid (0.152 g, 58%, 69% based on recovered starting material) and 0.05 g of starting material. R_f 0.3 (hexanes/EtOAc 2:1); mp 86-91 °C (CH_2Cl_2), $[\alpha]^{17}_D$ – 29.5 (*c* = 1.0, $CHCl_3$); IR (film, cm^{-1}) ν 2981, 2952, 2929, 2856, 1759, 1681, 1624, 1610, 1579, 1475, 1434, 1399, 1370, 1295, 1246, 1226, 1188, 1157, 1140, 1101, 1054; ^1H NMR (600 MHz, $CDCl_3$) δ 6.98 (t, *J* = 3.0 Hz, 1H), 6.07 - 6.04 (m, 2H), 4.73 - 4.68 (m, 1H), 4.41 - 4.37 (m, 1H), 4.21 (s, 3H), 4.14 (t, *J* = 8.1 Hz, 1H), 4.05 (dd, *J* = 8.4, 6.0 Hz, 1H), 1.60 (s, 9H), 1.48 (s, 3H), 1.32 (s, 3H), 0.95 (s, 9H), 0.15 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.7, 160.5,

153.9, 149.7, 145.7, 133.6, 127.3, 126.6, 111.4, 109.0, 100.0, 84.1, 79.8, 79.7, 77.0, 73.8, 60.5, 57.7, 27.7, 27.0, 25.9, 25.1, 18.2, −4.52, −4.97; HRMS (+EI) calcd for $C_{28}H_{41}N_2O_9Si$: 577.2581; found: 577.5204.

tert-Butyl (3a*S*,3b*R*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-6-hydroxy-2,2-dimethyl-5-oxo-3a,5,12,12a-tetrahydro[1,3]benzodioxolo[4,5-*h*][1,3]dioxolo[4,5-*b*]-1,6-naphthyridine-4(3b*H*)-carboxylate (543)

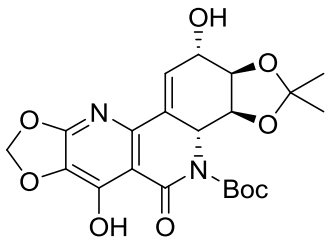


To a solution of **542** (0.050 g, 0.086 mmol) in dry DMF (5 mL) was added LiCl (0.010 g), followed by three cycles of freeze-pump-thaw. The reaction mixture was heated to 90 °C for 4.5 h. The reaction was then cooled to room temperature, diluted with distilled water (50 ml) and extracted with EtOAc (6 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The product was isolated by column chromatography (hexanes/EtOAc, 3:1) as a white crystalline solid (0.027 g, 56%).

mp 169-175 °C (EtOAc); R_f 0.9 (hexanes:EtOAc 2:1); $[\alpha]^{20}_D$ + 36.9 (*c* = 1.0, $CHCl_3$); IR (KBr, cm^{-1}) ν 3401, 2982, 2953, 2930, 2858, 1721, 1681, 1627, 1609, 1579, 1400, 1370, 1247, 1219, 1157, 1054, 835; ^1H NMR (600 MHz, $CDCl_3$) δ 12.70 (s, 1H), 7.02 (t, *J* = 3.0 Hz, 1H), 6.12 (d, *J* = 3.3 Hz, 2H), 4.77 - 4.72 (m, 1H), 4.39 (dt, *J* = 5.3, 2.5 Hz, 1H), 4.19 (t, *J* = 8.1 Hz, 1H), 4.08 (dd, *J* = 8.1, 5.8 Hz, 1H), 1.62 (s, 9H), 1.50 (s, 3H), 1.34 (s,

3H), 0.96 (s, 9H), 0.17 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.3, 162.2, 151.8, 149.8, 144.4, 133.6, 125.6, 111.6, 103.5, 101.1, 85.3, 79.4, 77.0, 73.6, 58.4, 27.6, 27.0, 25.8, 25.1, 18.2, −4.54, −4.98; HRMS (+EI) calcd for $C_{27}H_{38}N_2O_9Si$: 562.2347; found: 562.2317.

***tert*-butyl (3a*S*,3b*R*,12*S*,12a*R*)-6,12-dihydroxy-2,2-dimethyl-5-oxo-3a,5,12,12a-tetrahydro[1,3]benzodioxolo[4,5-*h*][1,3]dioxolo[4,5-*b*]-1,6-naphthyridine-4(3b*H*)-carboxylate (544)**

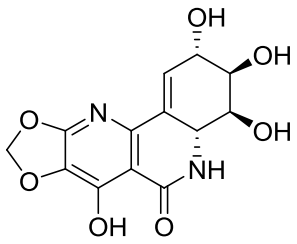


Pyridine **543** (24 mg, 0.043 mmol) was dissolved in THF (2.5 mL) and cooled to 0 °C. A solution of TBAF in THF (1 M, 0.10 mL, 0.10 mmol) was added dropwise over 1 min. The reaction mixture was stirred until total consumption of starting material was observed (TLC). The reaction mixture was quenched with NH_4Cl (sat. aq., 2 ml,) and extracted with EtOAc (6 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated. Compound **544** was isolated by column chromatography (EtOAc) as white crystalline solid (17 mg, 89%).

R_f 0.4 (hexanes:EtOAc 1:1); mp> 200°C (EtOAc); $[\alpha]^{20}_D$ − 32.4 (*c* = 0.35, MeOH); IR (KBr, cm^{-1}) ν 3396, 2983, 2933, 1762, 1680, 1609, 1609, 1479, 1434, 1400, 1372, 1297, 1227, 1157, 1140, 1104, 1075, 1054; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.59 (s, 1H), 7.02 (t, *J* = 3.0 Hz, 1H), 6.12 (d, *J* = 4.8 Hz, 2H), 4.78 - 4.69 (m, 1H), 4.48 - 4.40 (m, 1H),

4.33 (t, J = 8.1 Hz, 1H), 4.21 - 4.12 (m, 1H), 1.61 (s, 9H), 1.52 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 162.2, 151.4, 149.6, 144.0, 131.2, 126.2, 125.8, 112.0, 103.3, 101.2, 85.5, 79.2, 79.1, 77.0, 72.9, 58.6, 27.6, 27.0, 25.0; HRMS (+EI) calcd for $C_{22}H_{24}N_2O_9$: 448.1482; found: 448.1463.

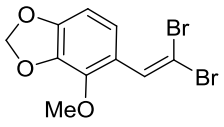
(2*S*,3*R*,4*S*,4a*R*)-2,3,4,7-Tetrahydroxy-3,4,4a,5-tetrahydrobenzo[*h*][1,3]dioxolo[4,5-*b*]-1,6-naphthyridin-6(2*H*)-one (5c)



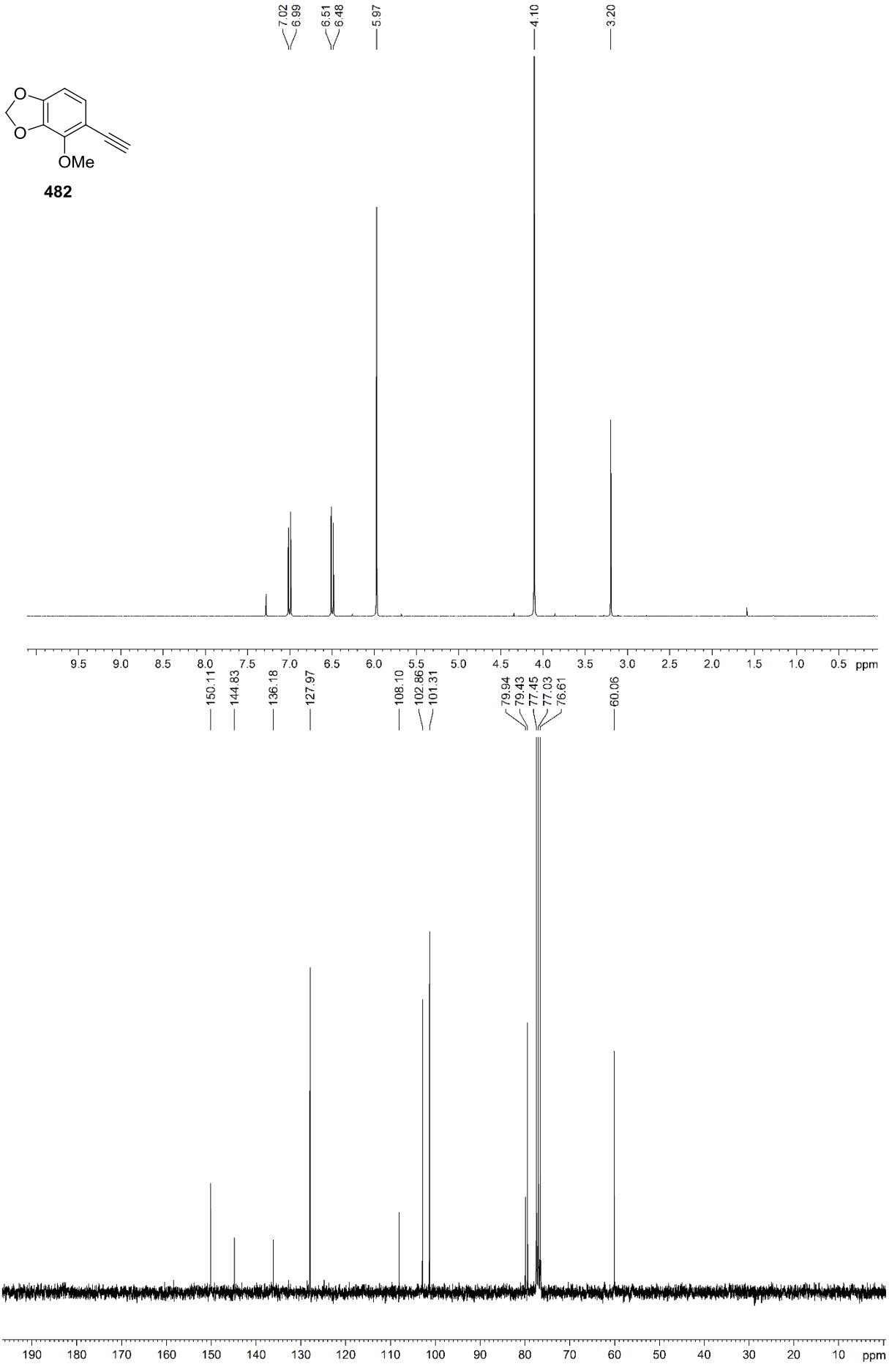
The Boc-protected amide **544** (0.024 g, 0.054 mmol) was dissolved in CH_2Cl_2 (4 mL) and cooled to 0 ˚C. Trifluoroacetic acid (0.2 mL 5% v/v H_2O) was added dropwise and the reaction was stirred until total consumption of starting material was observed (TLC). The reaction mixture was neutralized with excess of concetrated NH_3, evaporated and finally dried under high-vac. The final product was isolated by column chromatography on silica gel (deactivated by 10% of water, gradient CH_2Cl_2 - CH_2Cl_2:CH_3OH 50:1 - CH_2Cl_2:CH_3OH 20:1) as off-white crystalline compound (15 mg, 90%).

R_f 0.6 (CH_2Cl_2:MeOH 5:1); mp> 200°C (MeOH); $[\alpha]^{20}_D$ +4.1 (c =1, MeOH); IR (KBr, cm^{-1}) ν 3202, 3054, 2958, 2924, 2857, 1664, 1628, 1593, 1541, 1509, 1458, 1432, 1398, 1258, 1138, 1025, 802; ^{1}H NMR (600 MHz, CD_3OD) δ 6.66 (s, 1H), 6.13 (s, 2H), 4.48 (d, J = 8.5 Hz, 1H), 4.31 – 4.25 (m, 1H), 3.95 (s, 1H), 3.93 (d, J = 9.1 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 169.1, 161.7, 161.6, 146.6, 130.0, 125.7, 117.8, 115.9, 101.2, 73.0, 69.2, 69.2, 51.9.

6. Selected spectra.

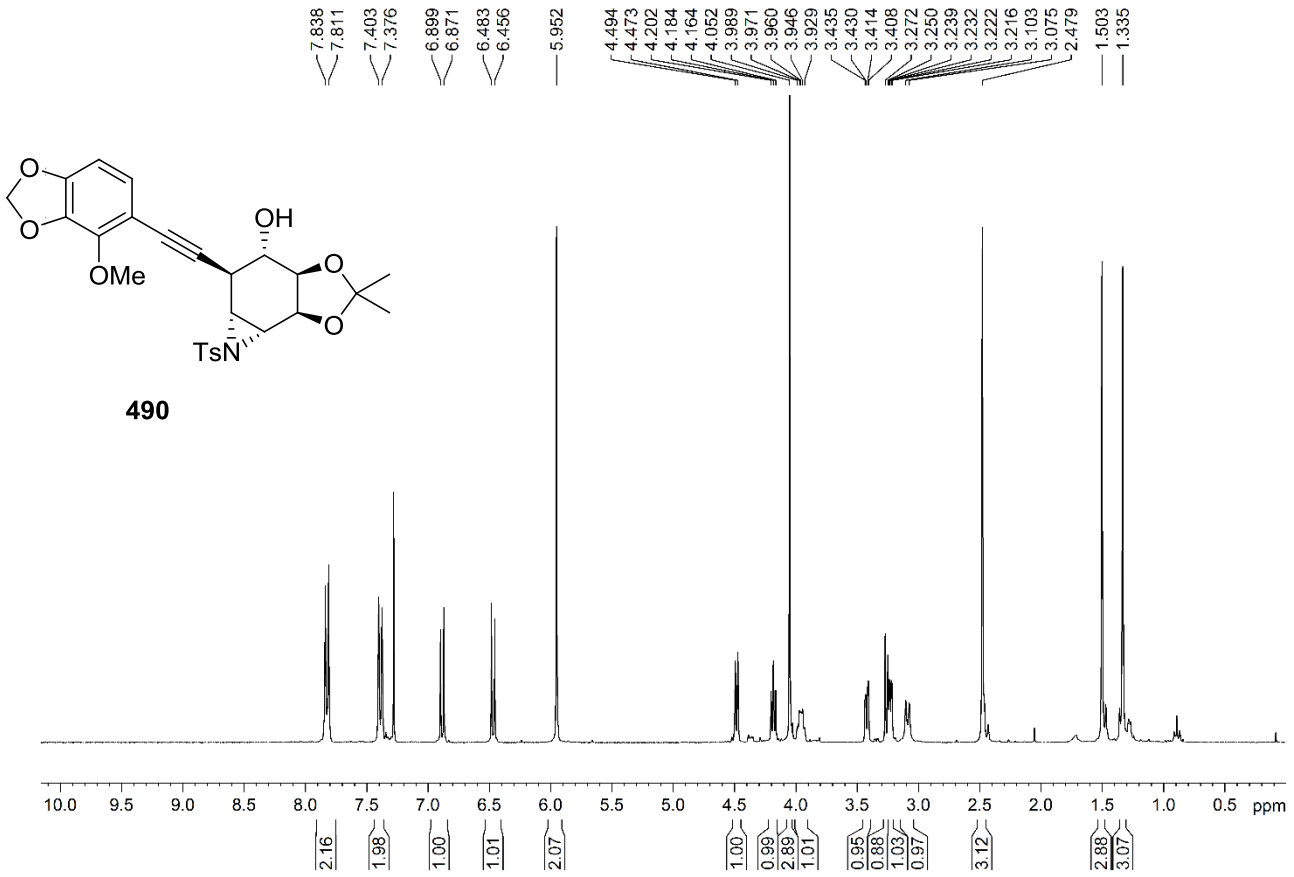


487


7.02
6.99
6.51
6.48
5.97
4.10
3.20
O
O
OMe
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
150.11
144.83
136.18
127.97
108.10
102.86
101.31
79.94
79.43
77.45
77.03
76.61
60.06
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

482

1D proton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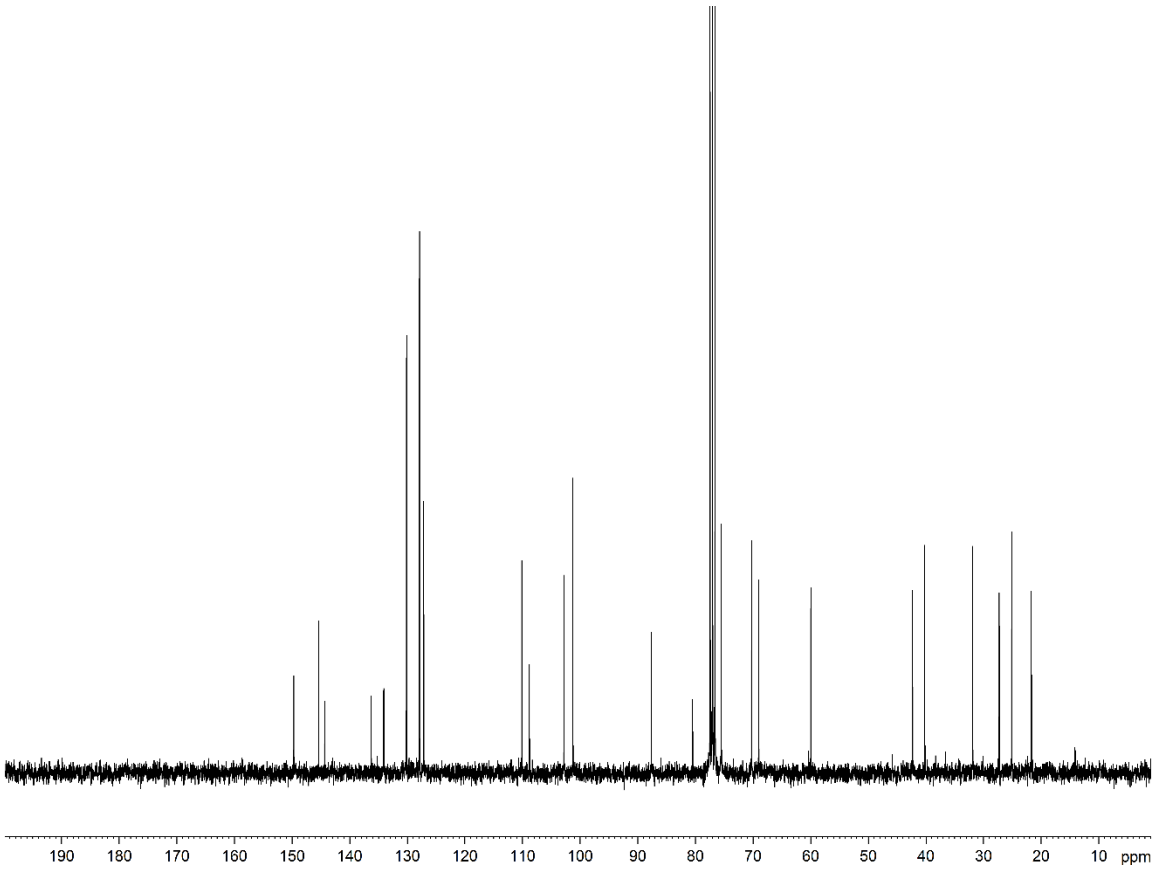
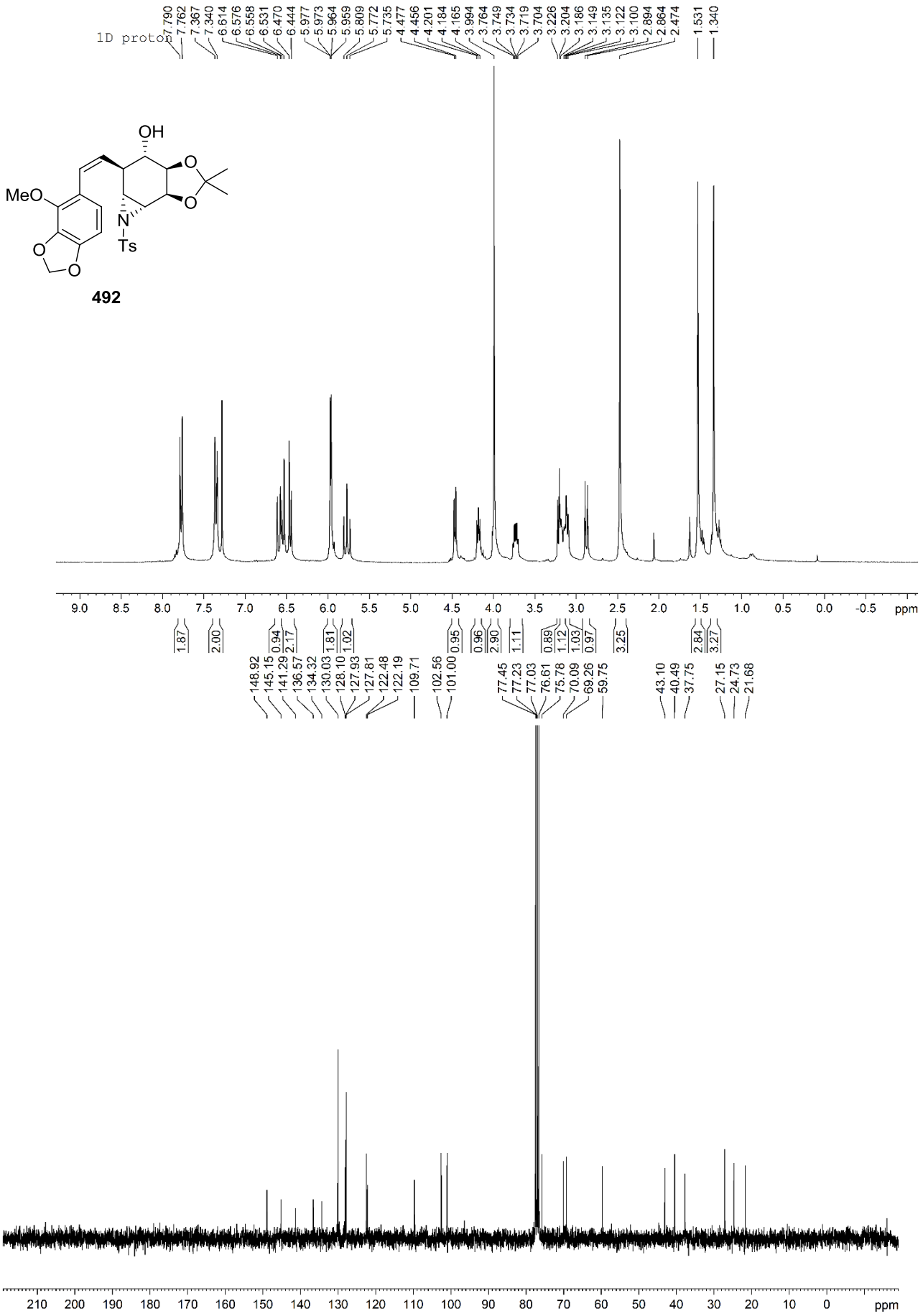
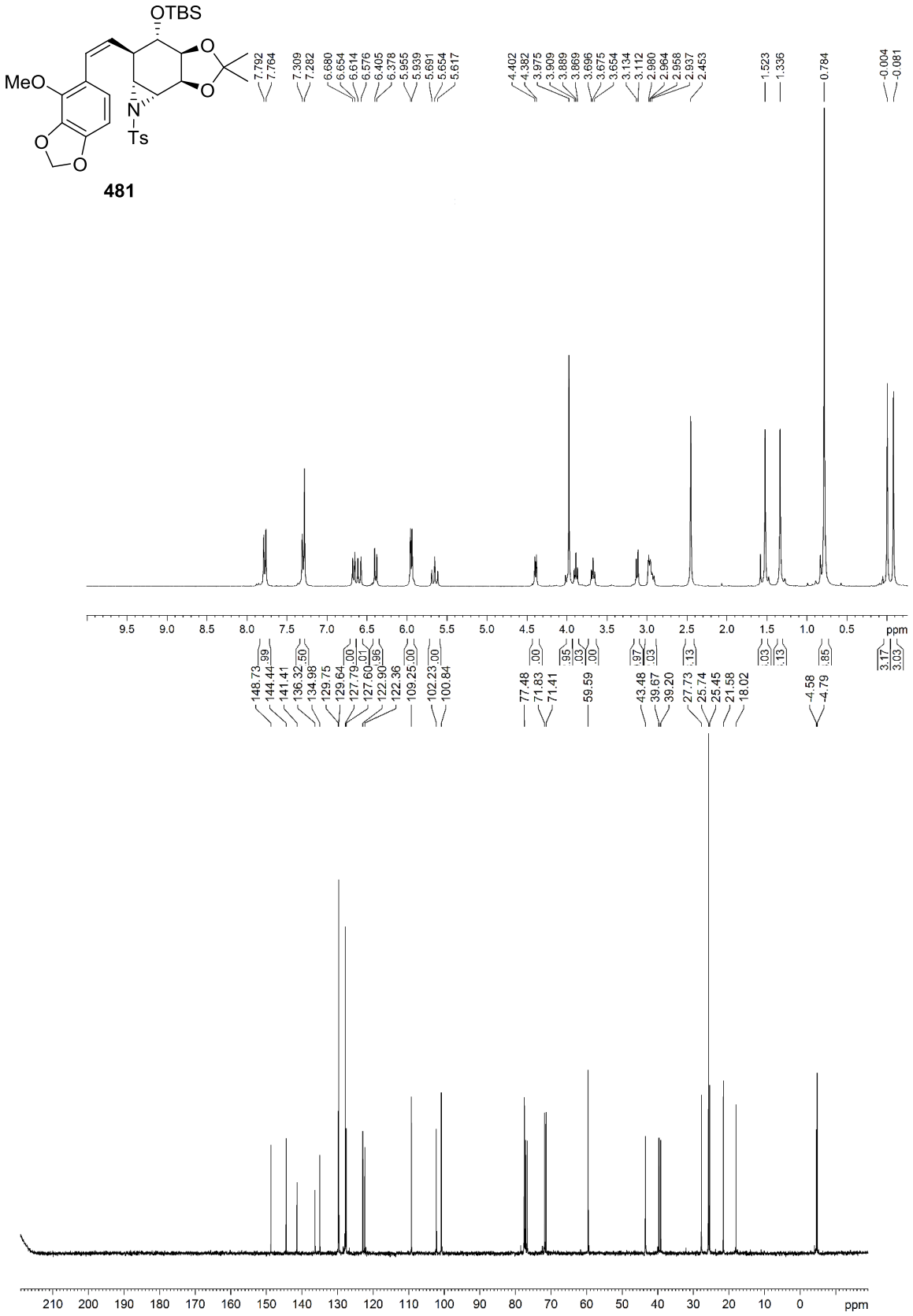
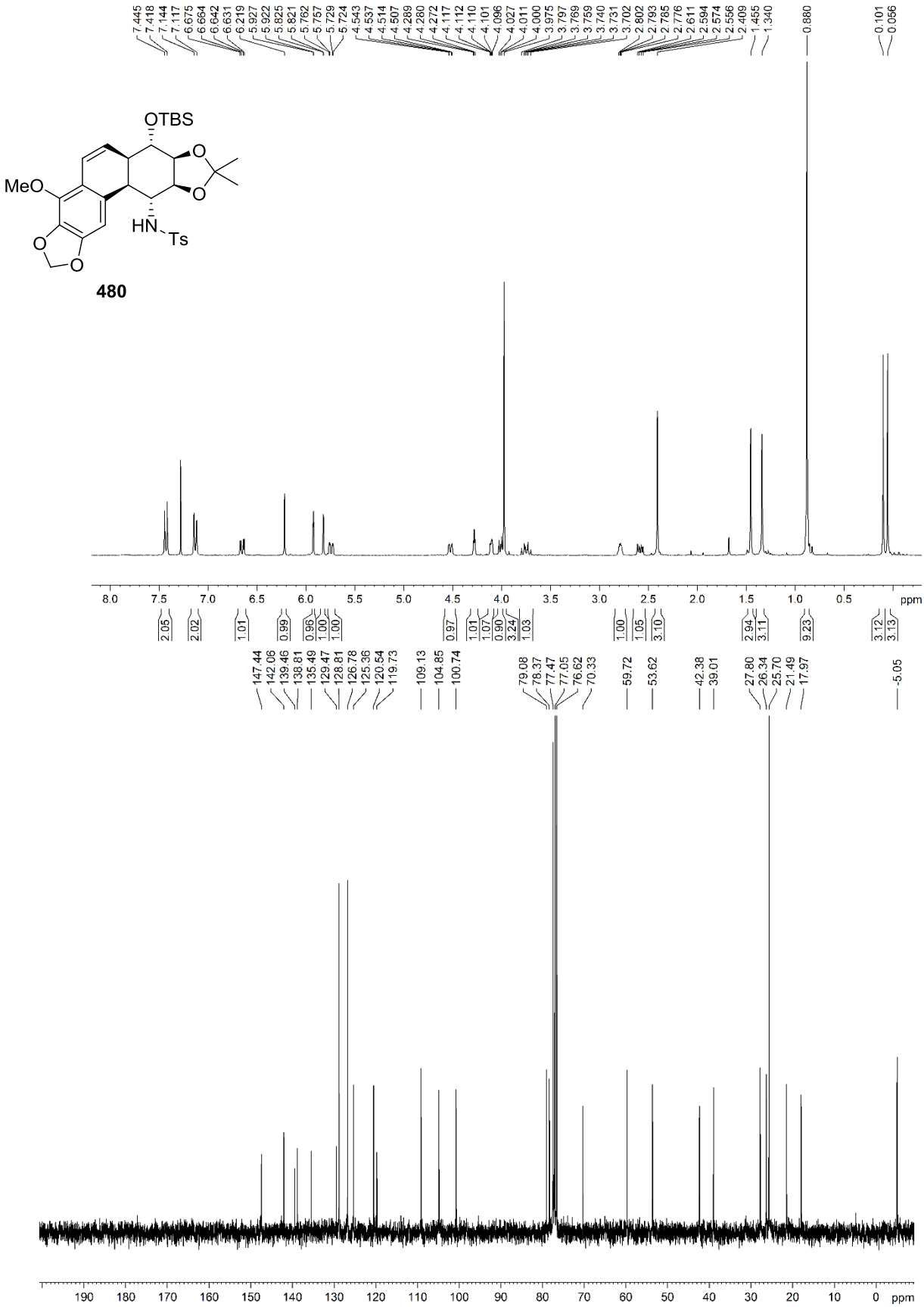
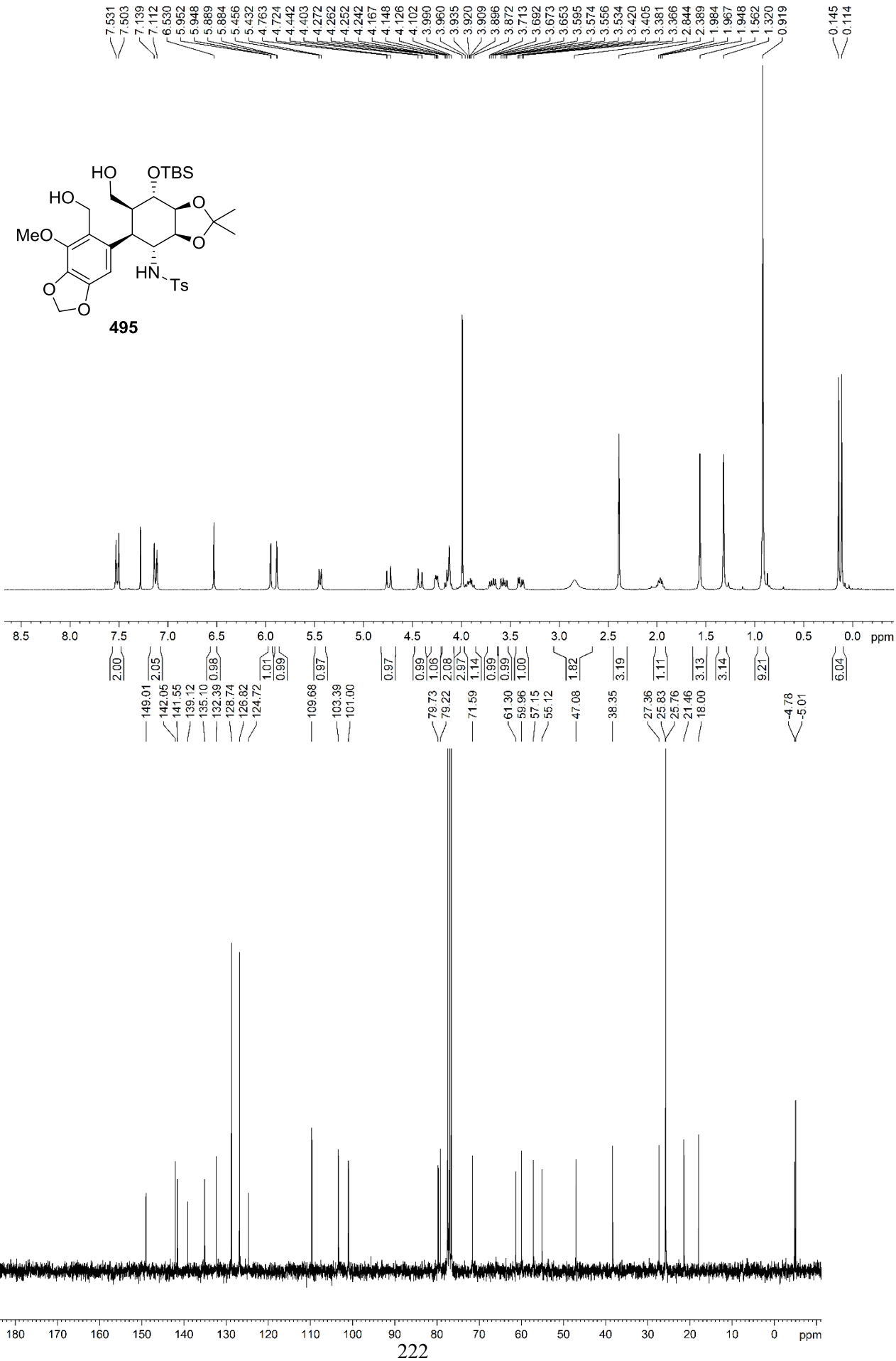
7.811
7.403
7.376
6.899
6.871
6.483
6.456
5.952
4.494
4.473
4.202
4.184
4.164
4.052
3.989
3.971
3.960
3.946
3.929
3.435
3.430
3.414
3.408
3.272
3.250
3.239
3.232
3.222
3.216
3.103
3.075
2.479
1.503
1.335
O
O
OMe
OH
O
O
TsN
490
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.16
1.98
1.00
1.01
2.07
1.00
0.99
2.89
1.01
0.95
0.88
1.03
0.97
3.12
2.88
3.07

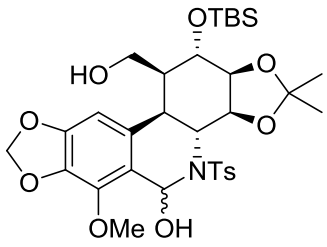

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


1D proton
7.790
7.762
7.367
7.340
6.614
6.576
6.558
6.531
6.470
6.444
5.977
5.973
5.964
5.959
5.809
5.772
5.735
4.477
4.456
4.201
4.184
4.165
3.994
3.764
3.749
3.734
3.719
3.704
3.226
3.204
3.186
3.149
3.135
3.122
3.100
2.894
2.864
2.474
1.531
1.340
OH
MeO
O
O
N
Ts
O
O
492
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 ppm
1.87
2.00
0.94
2.17
1.81
1.02
0.95
0.96
2.90
1.11
0.89
1.12
1.03
0.97
3.25
2.84
3.27
148.92
145.15
141.29
136.57
134.32
130.03
128.10
127.93
127.81
122.48
122.19
109.71
102.56
101.00
77.45
77.23
77.03
76.61
75.78
70.09
69.26
59.75
43.10
40.49
37.75
27.15
24.73
21.68
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


OTBS
MeO
O
O
N
Ts
O
O
481
7.792
7.764
7.309
7.282
6.680
6.654
6.614
6.576
6.405
6.378
5.955
5.939
5.691
5.654
5.617
4.402
4.382
3.975
3.909
3.889
3.869
3.696
3.675
3.654
3.134
3.112
2.980
2.964
2.958
2.937
2.453
1.523
1.336
0.784
0.004
0.081
ppm
148.73
144.44
141.41
136.32
134.98
129.75
129.64
127.79
127.60
122.90
122.36
109.25
102.23
100.84
77.48
71.83
71.41
59.59
43.48
39.67
39.20
27.73
25.74
25.45
21.58
18.02
-4.58
-4.79
ppm

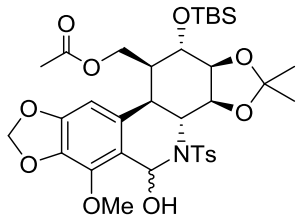

OTBS
MeO
O
O
HN
Ts
O
O
480
7.445
7.418
7.144
7.117
6.675
6.664
6.642
6.631
6.219
5.927
5.922
5.825
5.821
5.762
5.757
5.729
5.724
4.543
4.537
4.514
4.507
4.289
4.280
4.272
4.117
4.112
4.110
4.101
4.096
4.027
4.011
4.000
3.975
3.797
3.769
3.759
3.740
3.731
3.702
2.802
2.793
2.785
2.776
2.611
2.594
2.574
2.556
2.409
1.455
1.340
0.880
0.101
0.056
ppm
2.05
2.02
1.01
0.99
0.96
1.00
1.00
0.97
1.01
1.07
0.90
3.24
1.03
1.00
1.05
3.10
2.94
3.11
9.23
3.12
3.13
147.44
142.06
139.46
138.81
135.49
129.47
128.81
126.78
125.36
120.64
119.73
109.13
104.85
100.74
79.08
78.37
77.47
77.05
76.62
70.33
59.72
53.62
42.38
39.01
27.80
26.34
25.70
21.49
17.97
-5.05
ppm


HO
OTBS
HO
MeO
O
O
HN
Ts
O
O
495


OTBS
HO
O
O
O
O
NTs
OMe OH

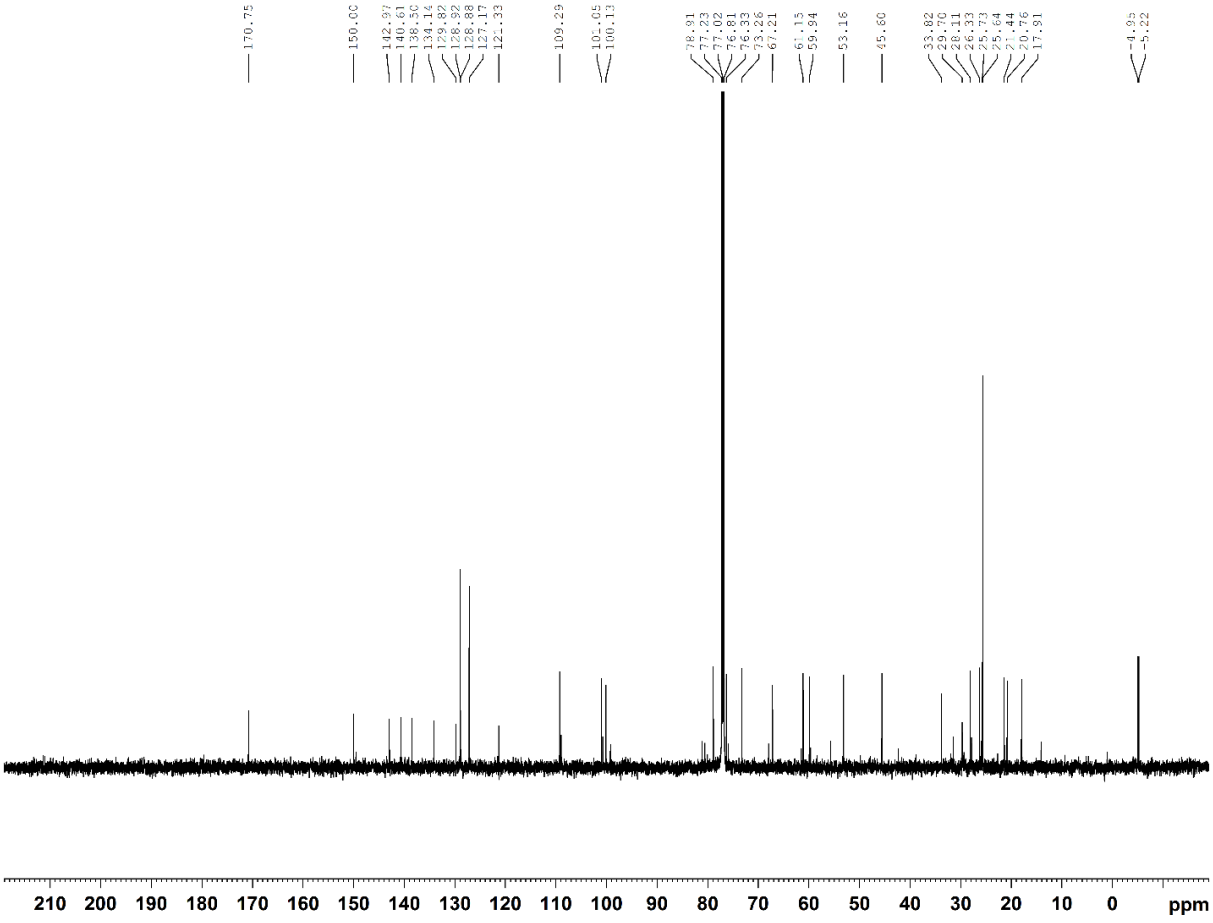
496

7.583
7.556
7.090
7.063
6.669
6.654
6.095
5.929
5.925
5.919
5.914
5.301
5.282
5.268
5.250
4.393
4.382
4.371
4.257
4.237
4.223
4.214
4.180
4.116
3.770
3.740
3.710
3.398
3.374
3.358
2.923
2.906
2.880
2.862
2.342
2.185
2.171
2.158
2.145
2.131
1.784
1.444
1.378
0.921
0.151
0.108
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
2.03 2.05 1.00 0.98 2.00 1.00 1.04 2.08 3.07 1.04 2.00 1.04 3.10 1.04 2.19 3.00 3.03 8.98 3.17 3.14
150.00
142.91
140.54
138.40
133.93
130.06
128.87
127.24
121.26
109.36
100.97
100.20
78.92
77.44
77.22
77.02
76.59
76.38
73.52
68.17
59.88
59.38
53.19
48.10
34.43
27.89
26.16
25.71
21.42
17.93
-4.74
-5.08
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


OTBS
O
O
O
O
O
O
NTs
OMe OH

497

7.571
7.558
7.088
7.074
6.659
6.119
5.941
5.938
5.327
5.318
5.310
5.302
4.371
4.223
4.215
4.142
4.124
4.112
4.106
4.089
4.083
4.066
3.922
3.915
3.904
3.896
3.050
2.925
2.917
2.903
2.695
2.348
1.929
1.471
1.379
0.933
0.152
0.114
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm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142.97
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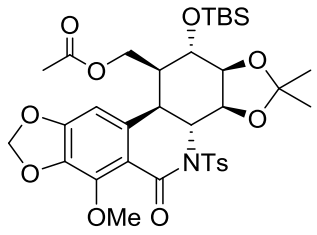
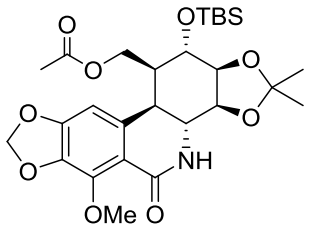
134.14
129.82
128.92
128.88
127.17
122.33
109.29
101.05
100.13
78.91
77.23
77.02
76.81
76.33
73.26
67.21
61.13
59.94
53.16
45.60
33.82
29.76
28.11
26.33
25.73
25.64
21.44
20.76
17.91
-4.93
-5.22
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm



498


OTBS
O
O
O
O
O
O
NH
OMe O

499

7.283
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6.058
6.056
6.012
6.010
5.921
4.569
4.566
4.233
4.221
4.189
4.181
4.158
4.150
4.145
4.136
4.066
3.430
3.417
3.407
3.393
3.320
3.314
3.297
3.291
2.655
2.651
2.646
2.106
1.464
1.387
0.907
0.159
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
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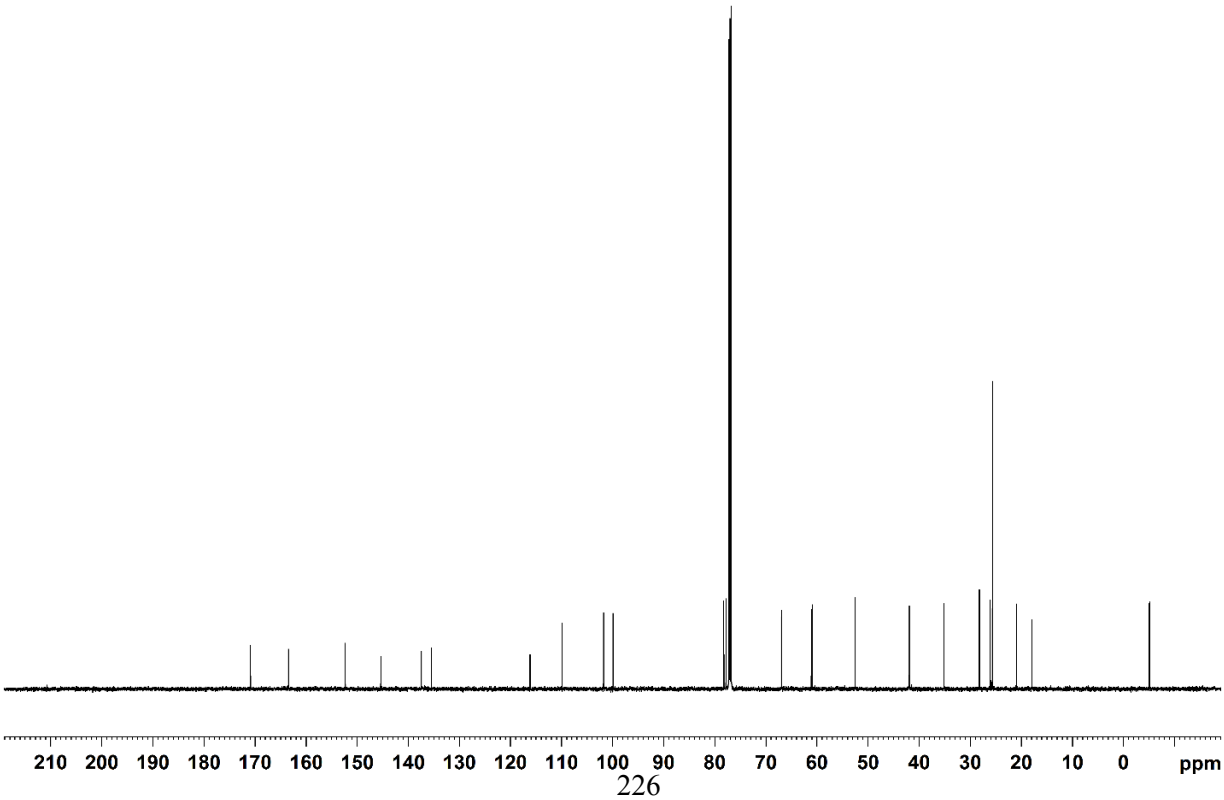
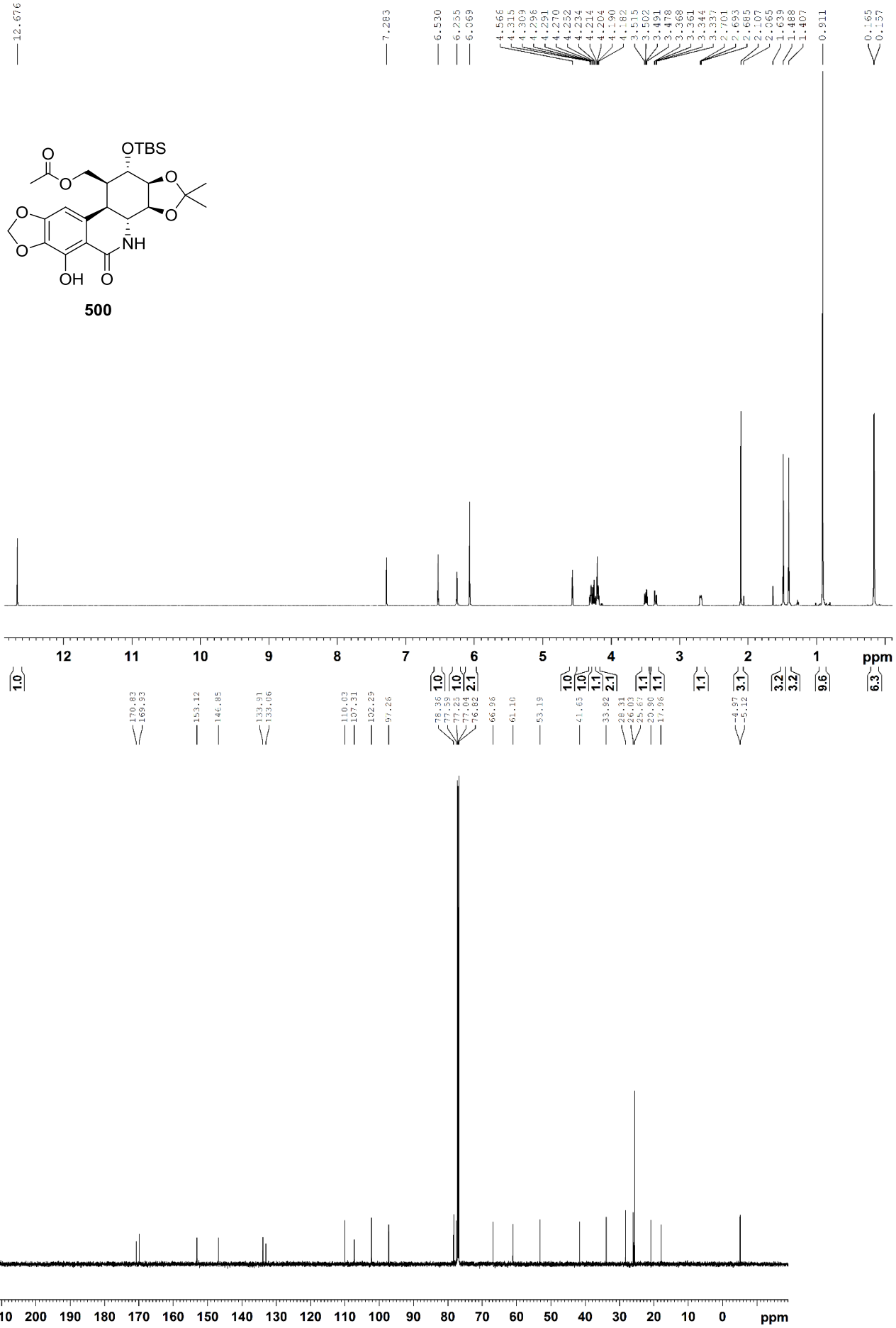

170.90
163.47
152.36
145.40
137.46
133.48
116.27
109.91
101.78
99.92
78.25
77.82
77.26
77.04
76.83
66.96
61.11
60.92
52.51
41.94
35.14
28.21
26.07
25.69
20.92
17.95
-4.96
-5.14
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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O
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O
O
NH
OH
O
500
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6.530
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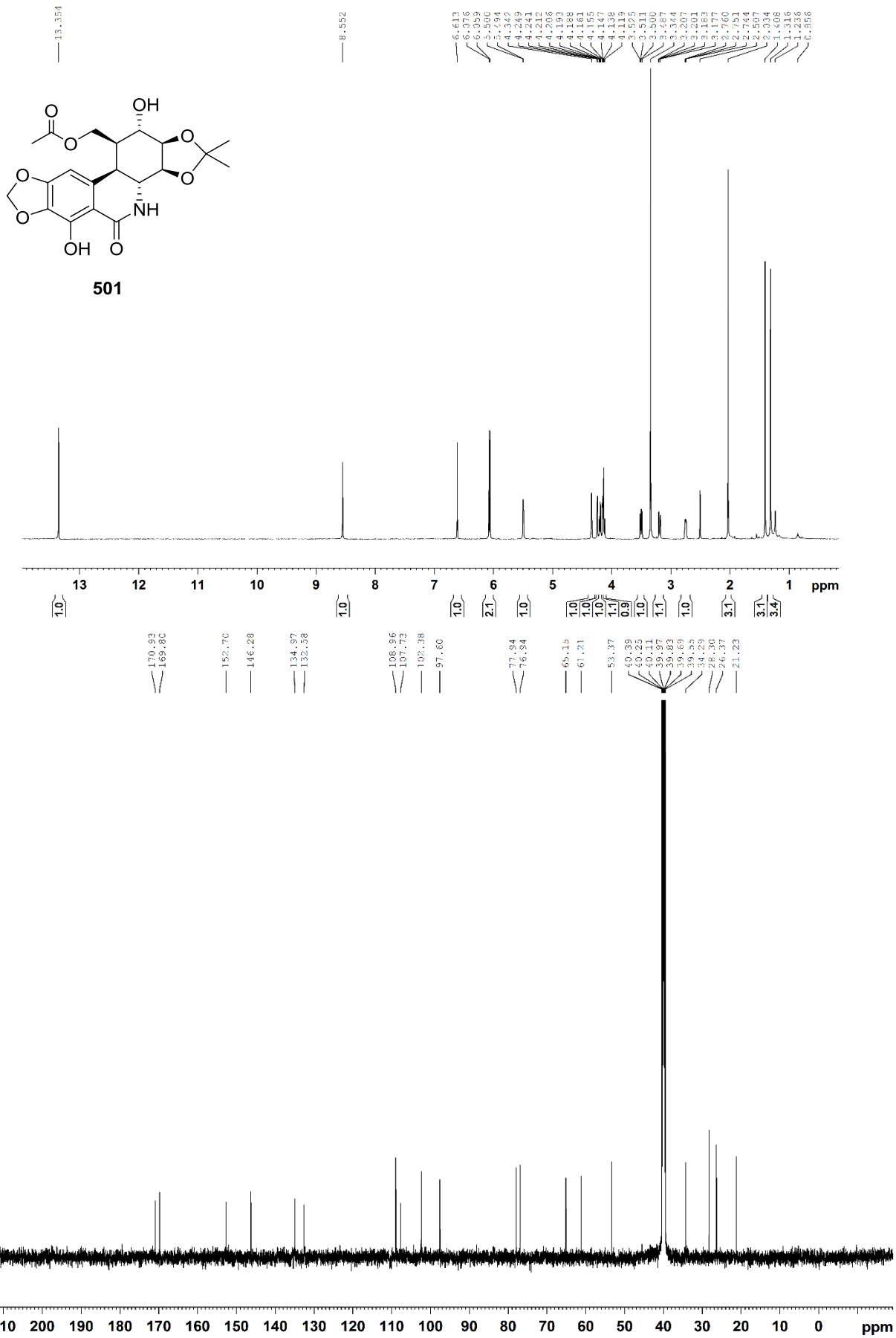
6.369
4.566
4.315
4.309
4.296
4.291
4.270
4.252
4.234
4.214
4.204
4.190
4.182
3.515
3.502
3.491
3.478
3.368
3.361
3.344
3.337
2.701
2.693
2.685
2.107
2.065
1.639
1.488
1.467
0.911
0.165
0.157
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.0
1.0
1.0
2.1
1.0
1.0
1.1
2.1
1.1
1.1
1.1
3.1
3.2
3.2
9.6
6.3
170.83
169.93
153.12
146.85
133.91
133.06
110.03
107.31
102.29
97.26
78.36
77.59
77.25
77.04
76.82
66.96
61.10
53.19
41.65
33.92
28.31
26.03
25.67
20.90
17.96
-4.97
-5.12
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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4.241
4.212
4.206
4.193
4.188
4.161
4.155
4.147
4.138
4.119
3.675
3.511
3.500
3.467
3.344
3.207
3.201
3.163
3.177
2.760
2.751
2.744
2.697
2.504
2.408
1.536
1.516
6.856
ppm
170.93
169.80
152.70
146.28
134.97
132.58
108.96
107.73
102.38
97.60
77.94
76.94
65.15
61.21
53.37
40.39
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39.97
39.83
39.69
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34.29
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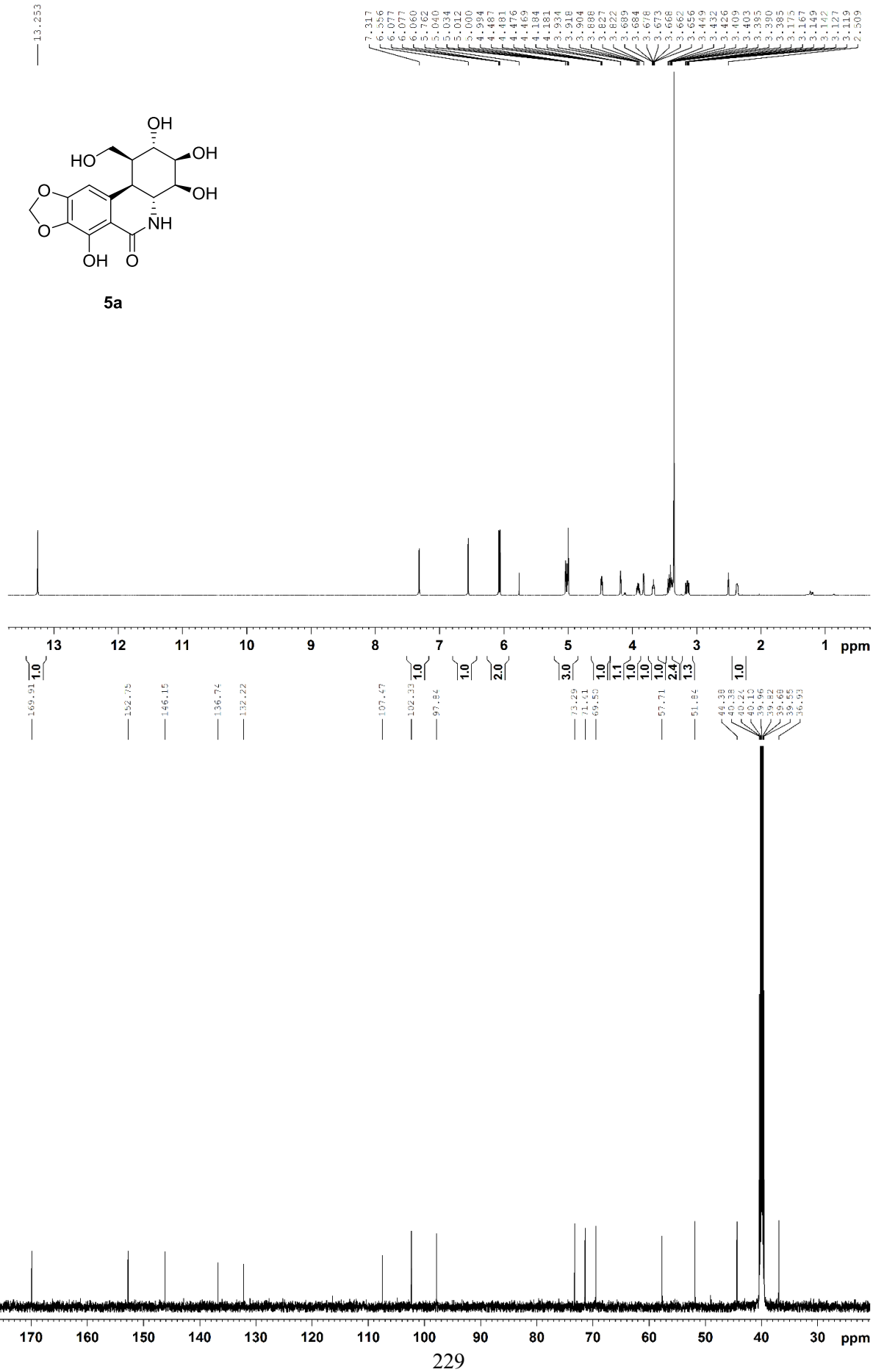
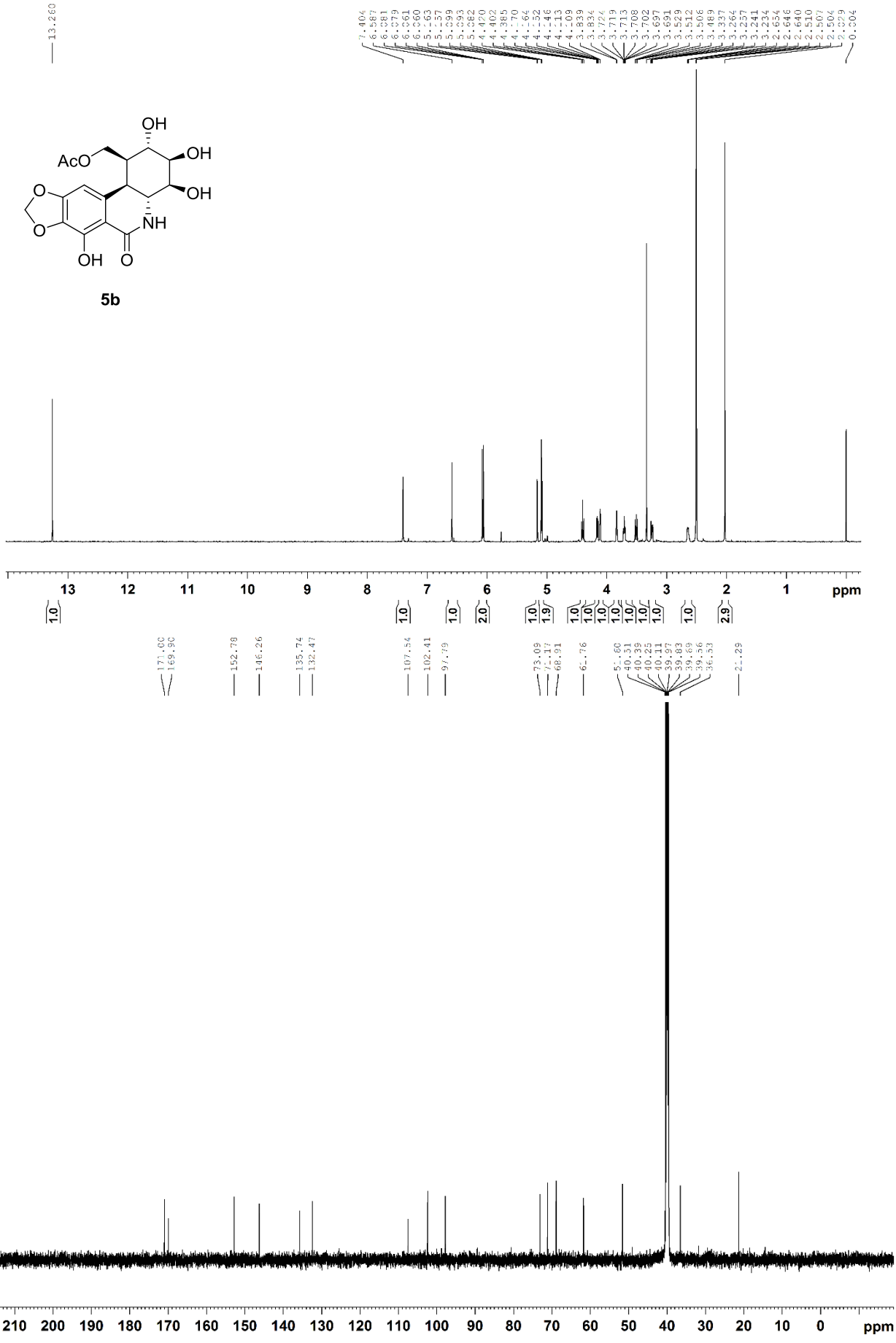
26.37
21.23
ppm

501


OH
HO
OH
OH
O
O
NH
OH
O
5a
ppm
ppm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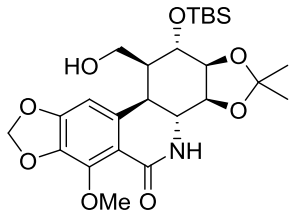
OH
O
O
NH
OH
O
5b
ppm
ppm


OTBS
HO
O
O
O
O
NH
OMe
O

479

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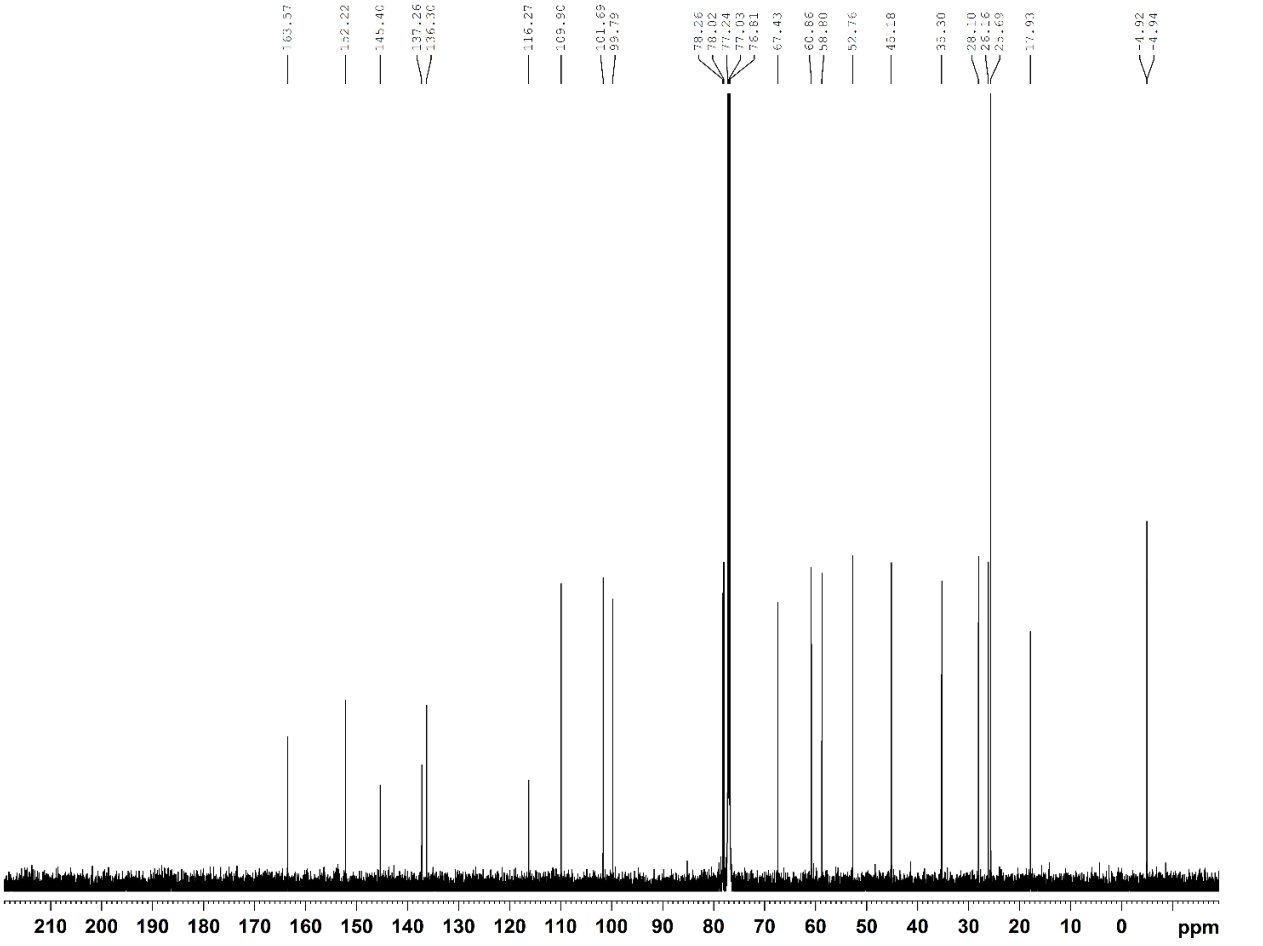
6.607
6.054
5.998
5.864
4.676
4.200
4.192
4.149
4.141
4.136
4.127
4.076
3.969
3.958
3.951
3.942
3.935
3.925
3.674
3.606
3.503
3.489
3.480
3.466
3.291
3.285
3.268
3.262
2.534
2.528
2.522
2.066
1.910
1.665
1.456
1.398
1.291
1.279
1.267
0.934
0.913
0.200
0.179
10
9
8
7
6
5
4
3
2
1
ppm
1.0
1.0
1.0
1.0
1.0
1.0
1.0
1.2
2.9
1.0
1.0
1.0
1.0
1.0
3.0
3.0
9.2
6.0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152.22
145.40
137.26
136.36
116.27
109.90
101.69
99.79
78.26
78.02
77.24
77.03
76.81
67.43
60.86
58.80
52.76
45.18
35.30
28.10
26.16
25.69
17.93
-4.92
-4.94
210
200
190
180
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140
130
120
110
100
90
80
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60
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30
20
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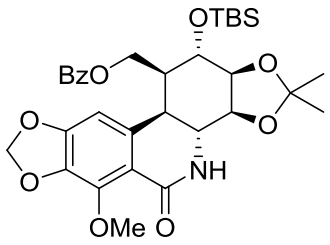
OTBS
BzO
O
O
O
O
NH
OMe O

503

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8.050
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7.607
7.583
7.502
7.476
7.451
6.744
6.007
6.000
4.676
4.570
4.533
4.498
4.482
4.470
4.445
4.433
4.203
4.178
4.161
4.074
3.567
3.542
3.521
3.496
3.403
3.392
3.357
3.346
2.856
2.843
2.830
1.503
1.396
0.904
0.150
ppm
166.37
163.58
152.38
145.43
135.61
133.16
129.85
129.60
128.41
116.15
109.96
101.75
99.95
78.26
77.89
77.45
77.03
76.60
67.19
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26.08
25.65
17.92
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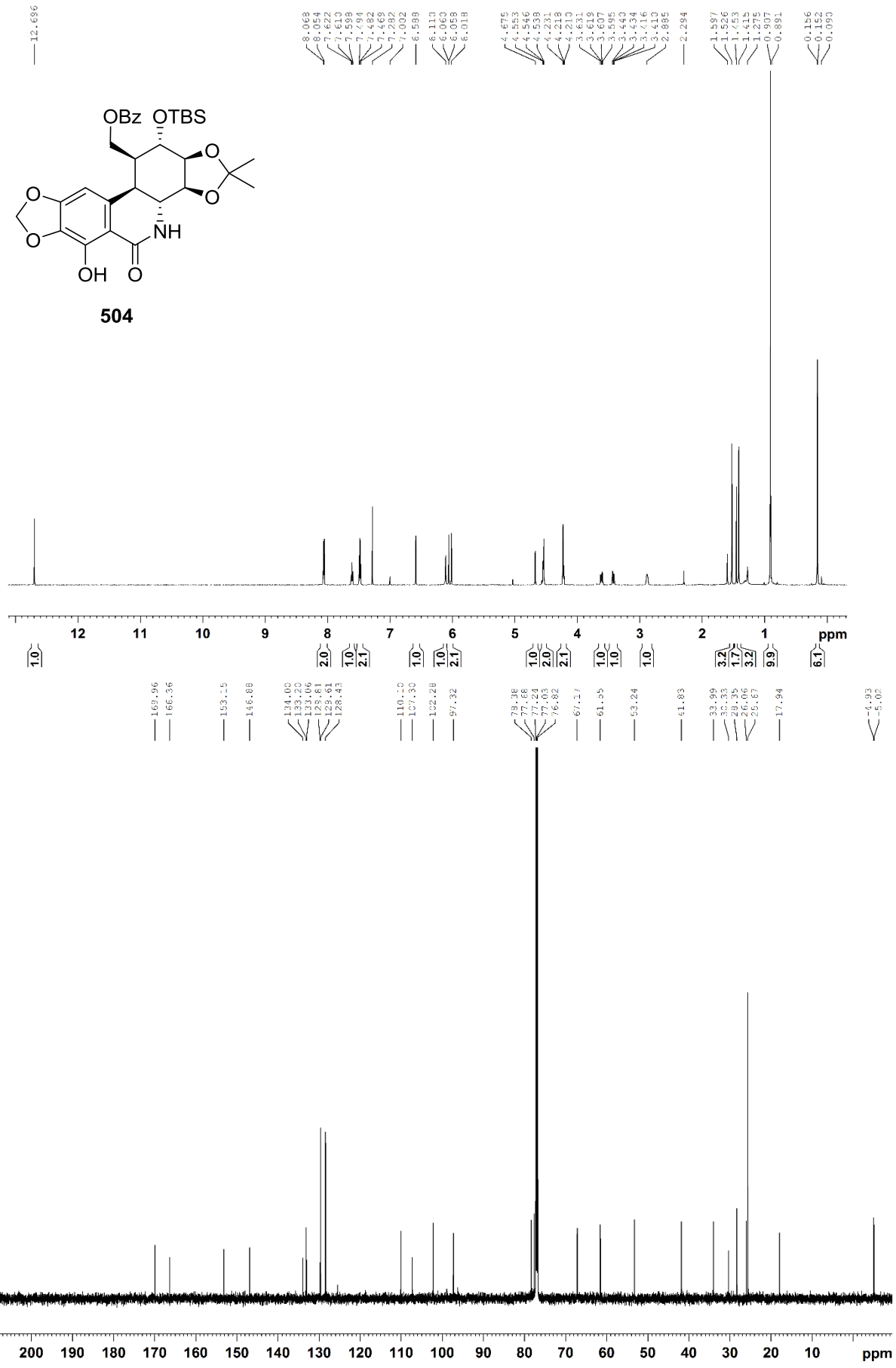
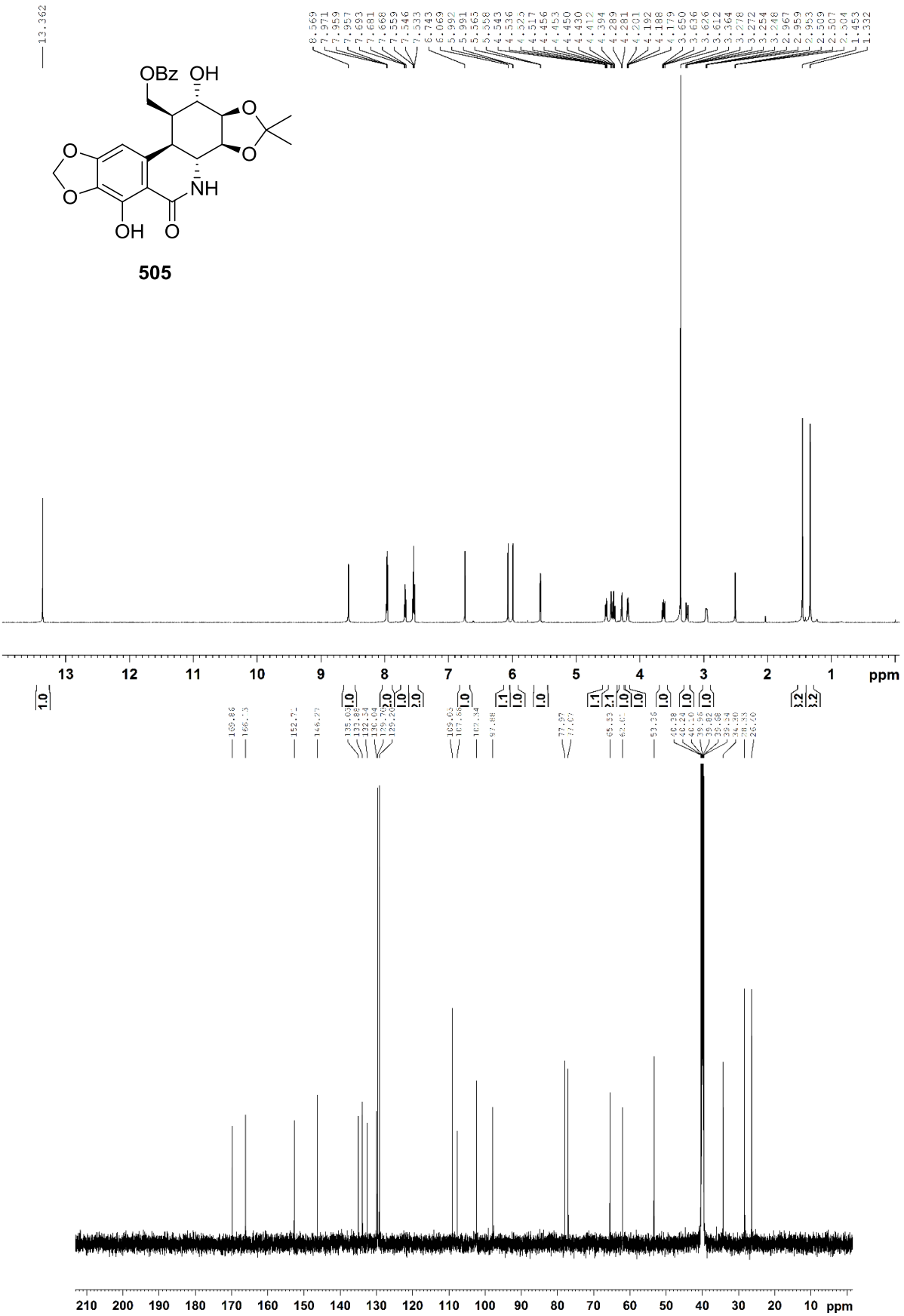
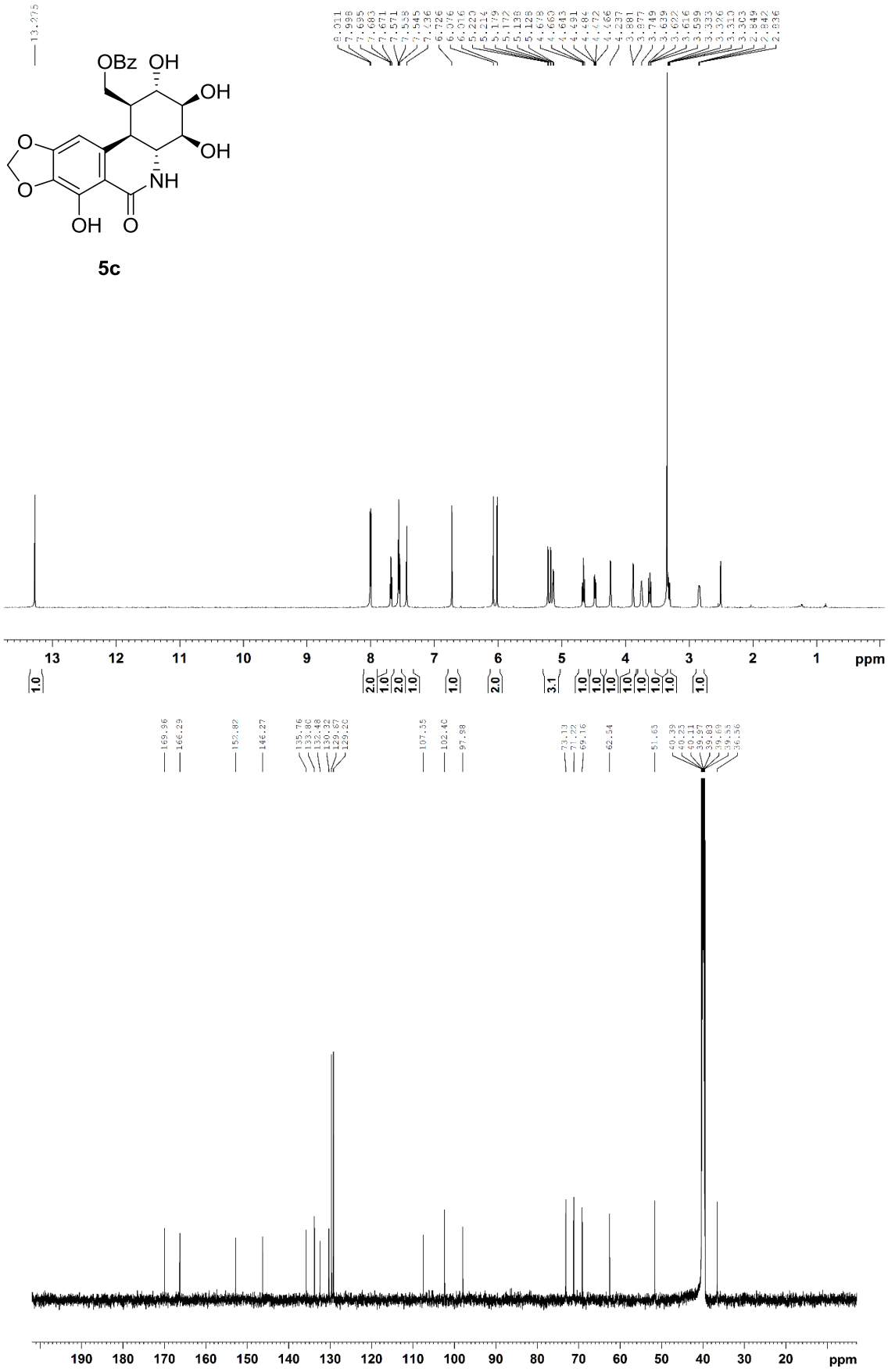
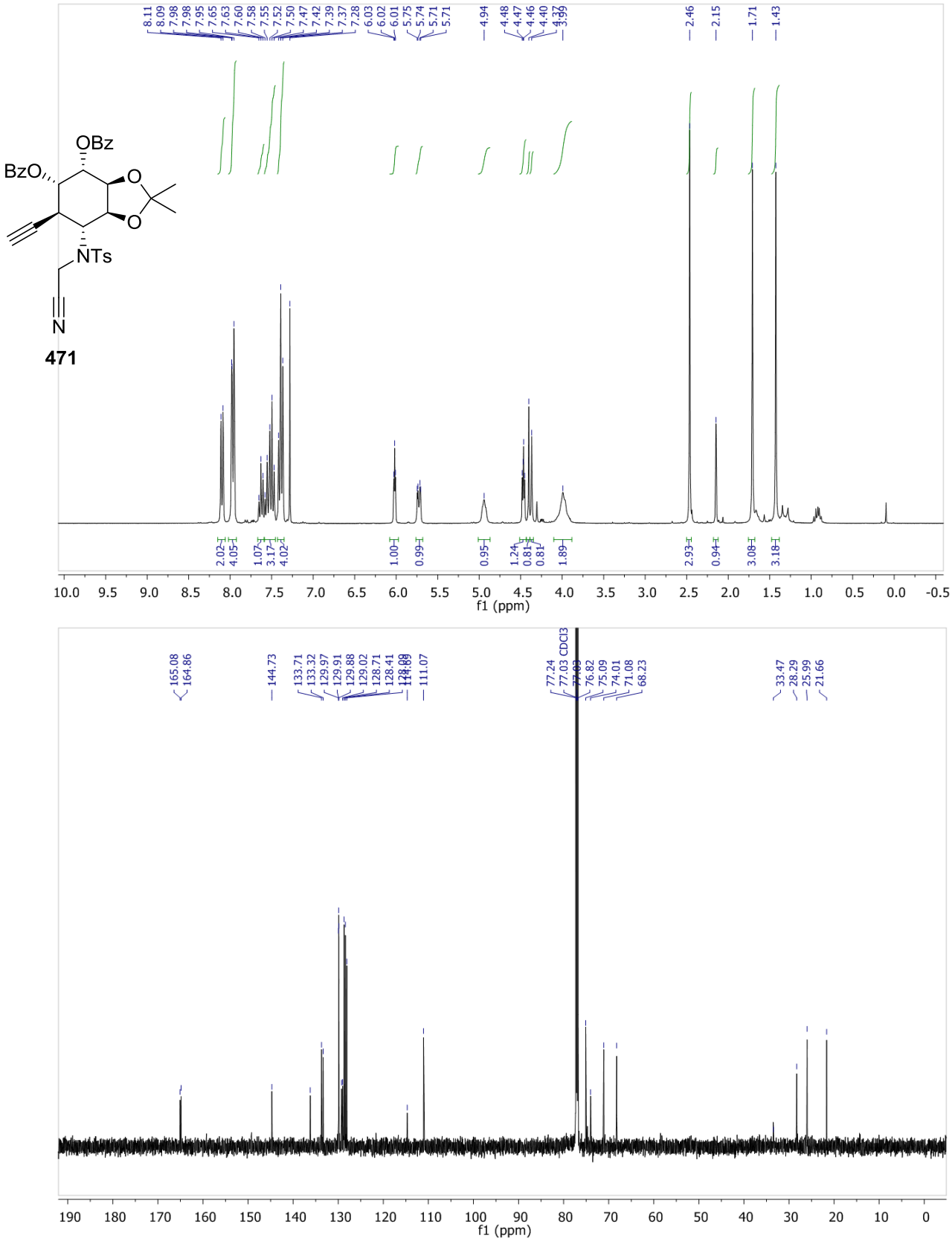
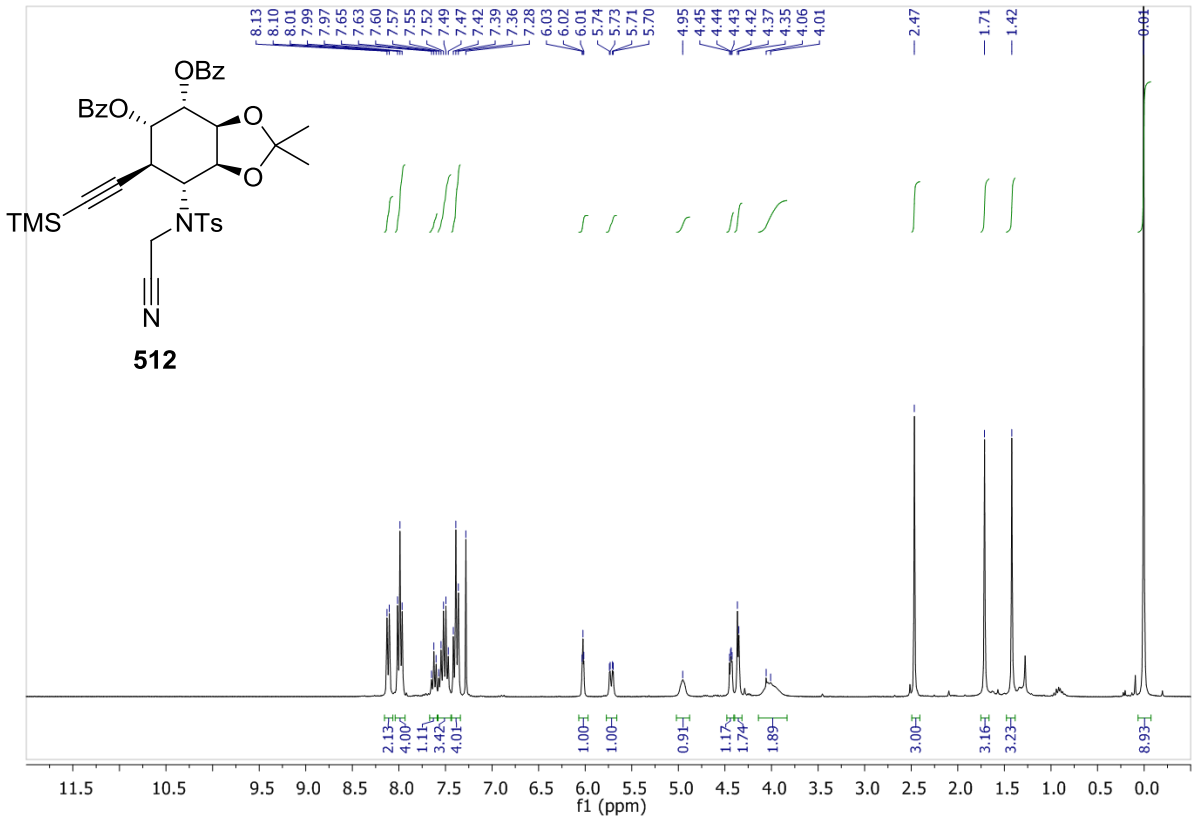
ppm

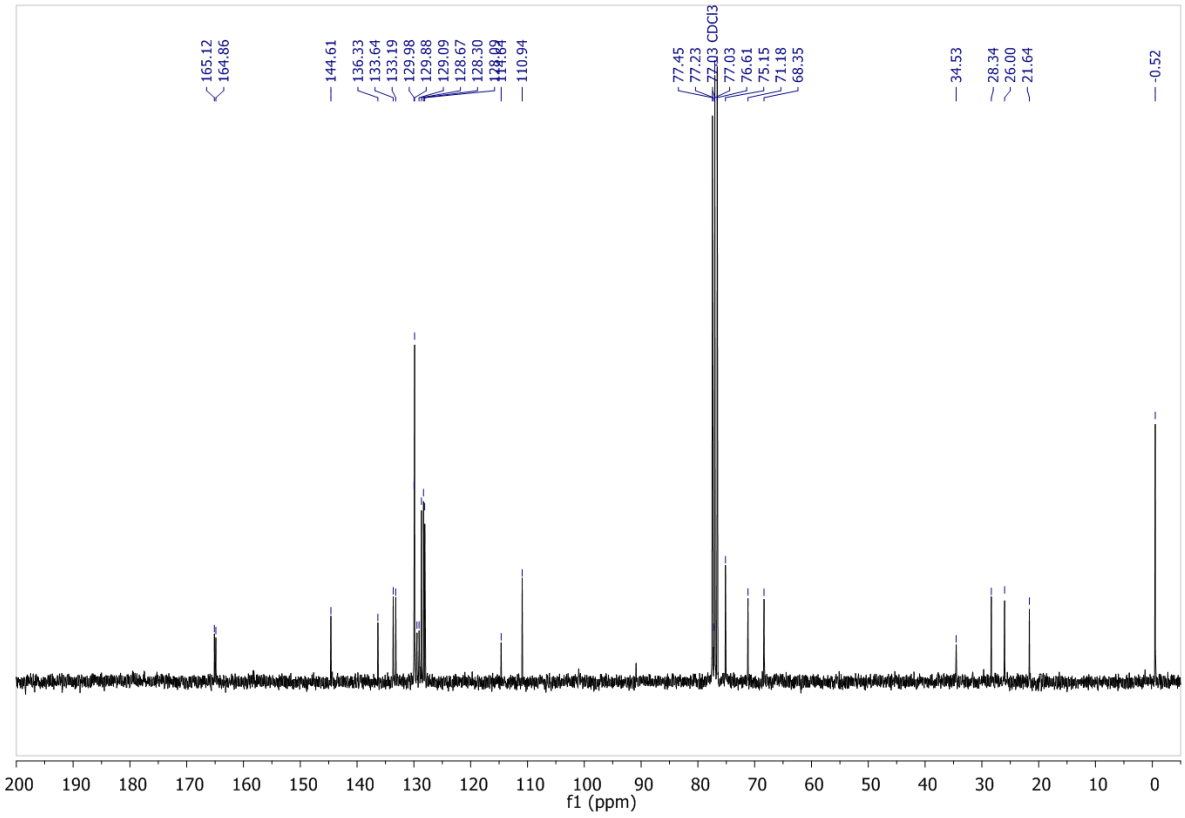

OBz
OTBS
O
O
O
O
NH
OH
O
504
ppm
ppm

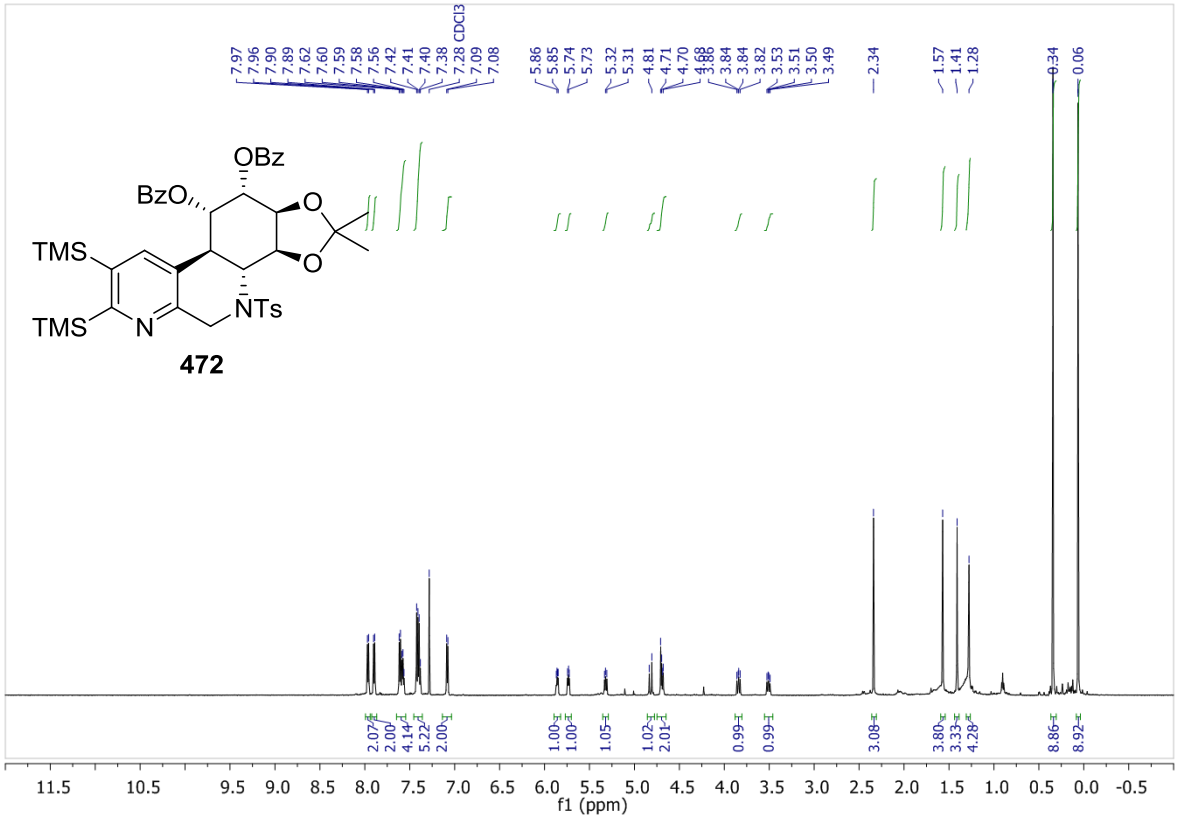

OBz
OH
O
O
O
O
NH
OH
O
505
ppm
ppm

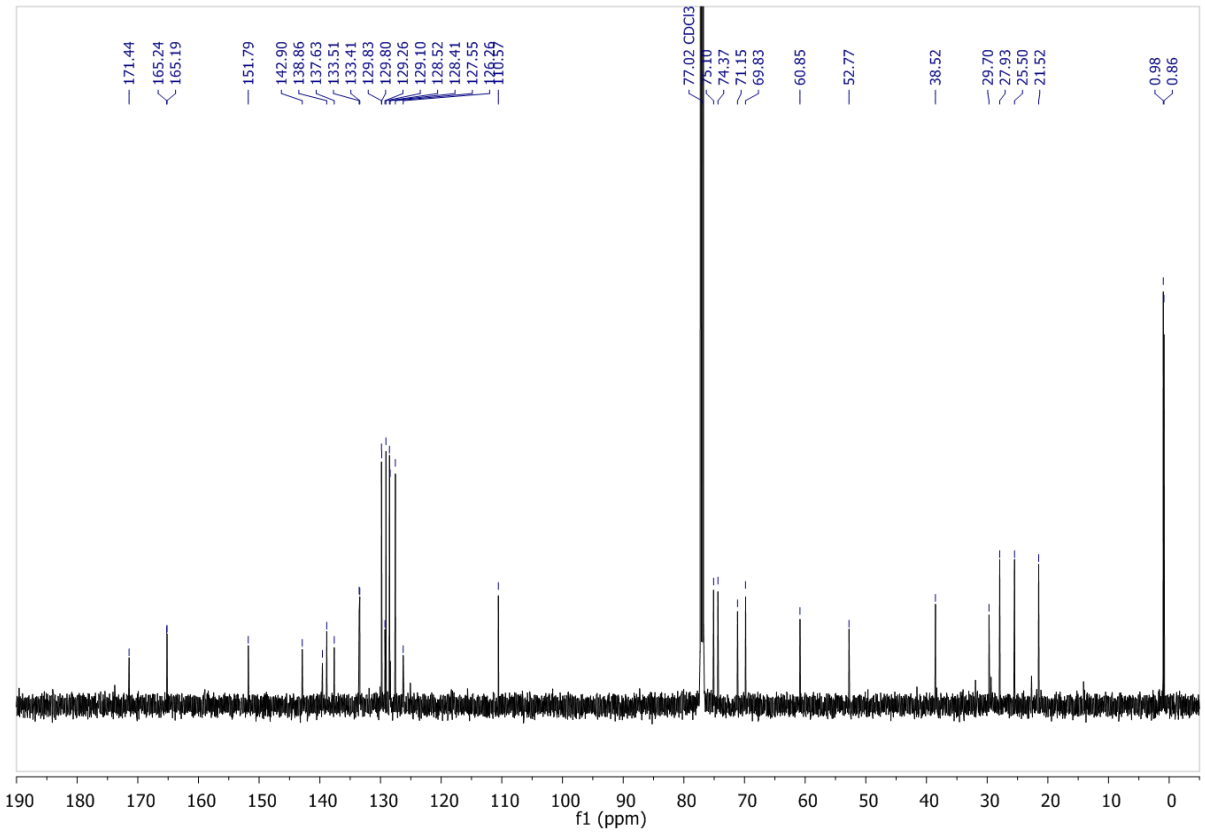

OBz
OH
OH
OH
O
O
NH
OH
O
5c
ppm
ppm

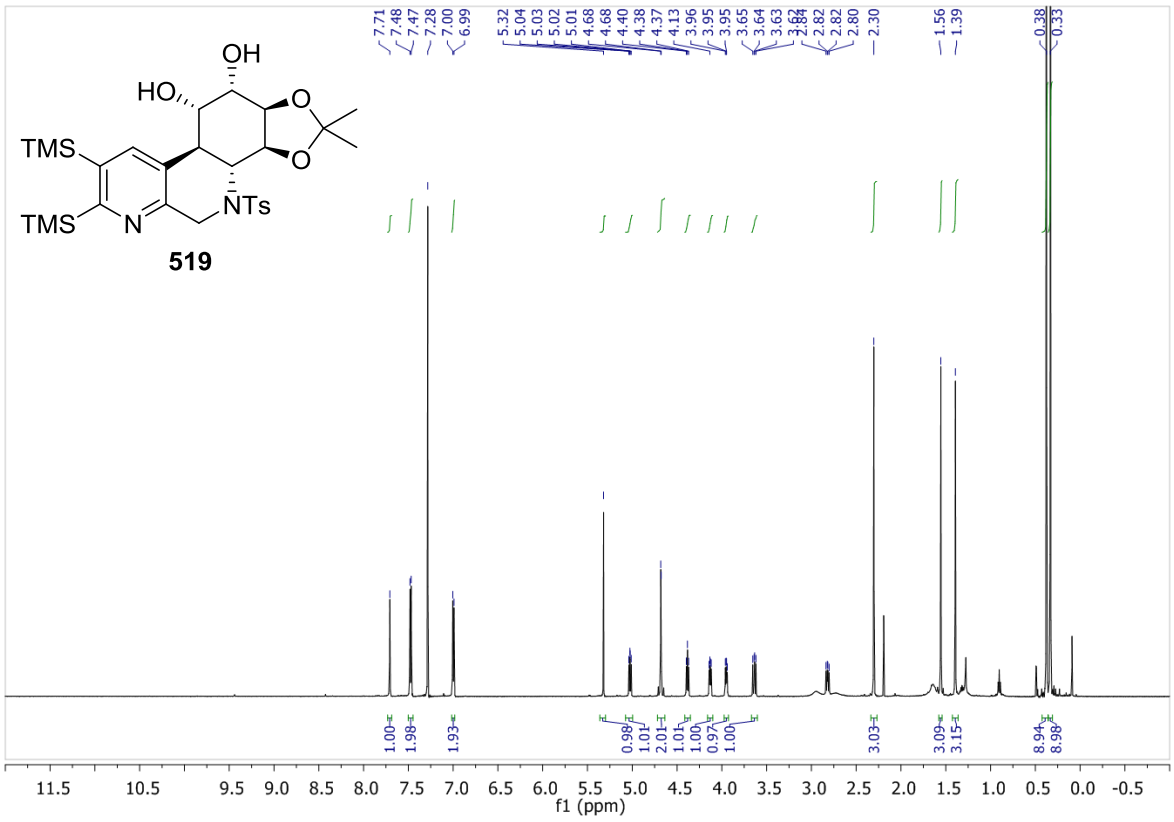

OBz
BzO
O
O
NTs
N
471
f1 (ppm)
f1 (ppm)

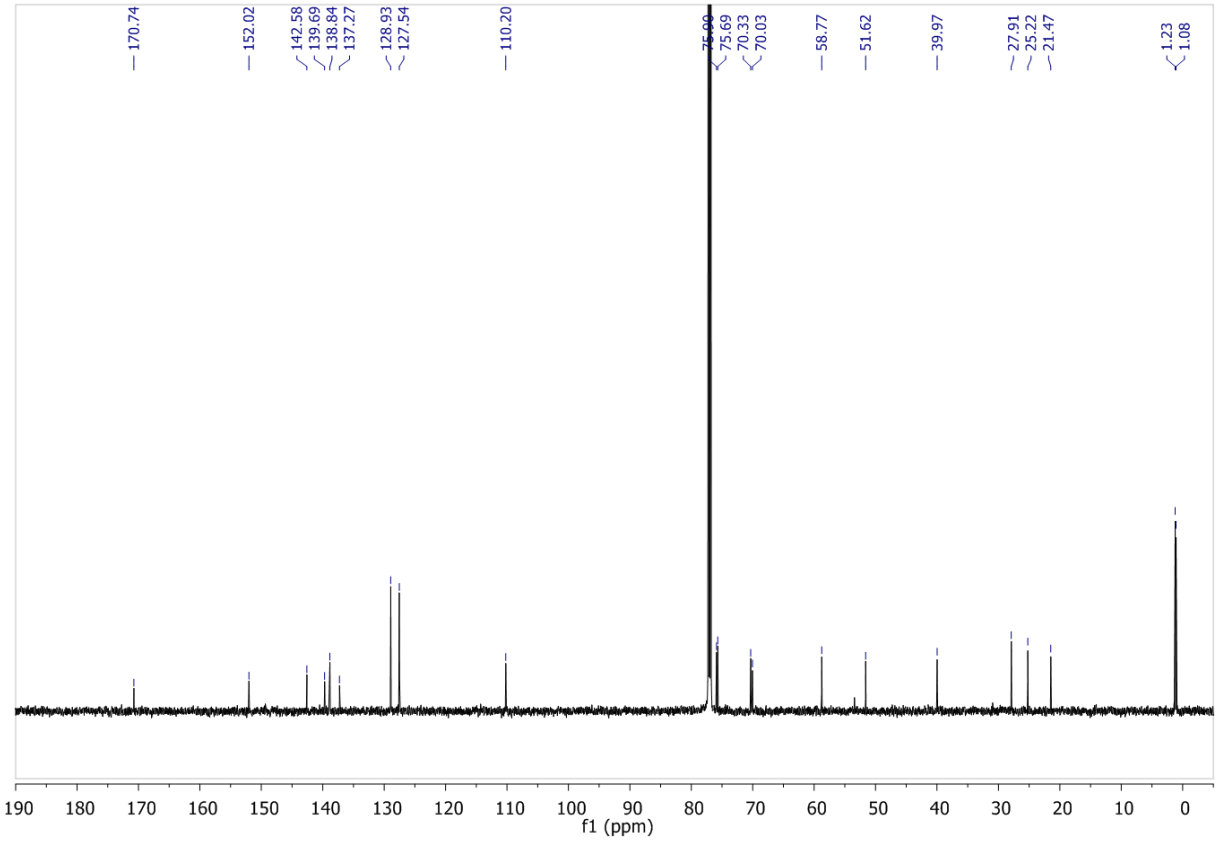

OBz
BzO
O
O
TMS
NTs
N
512
f1 (ppm)

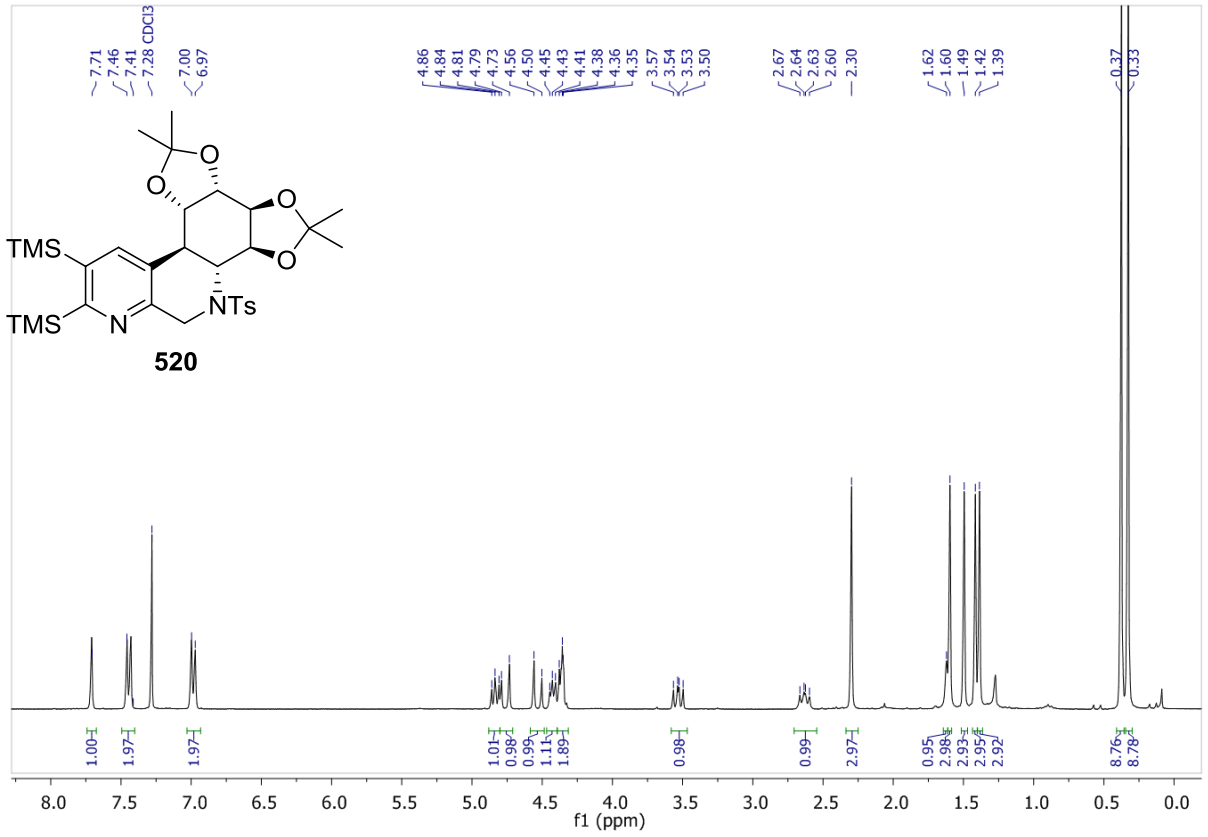

CDCl3
f1 (ppm)

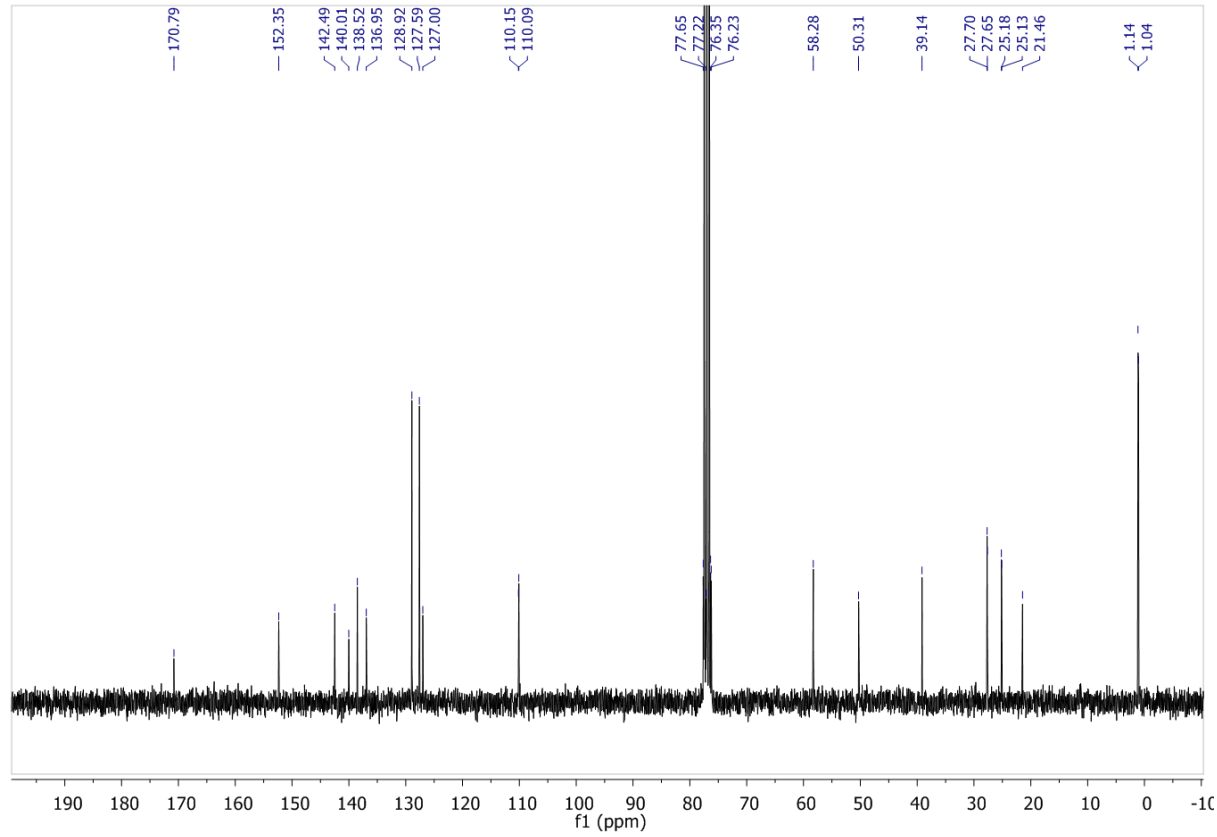

OBz
BzO
O
O
TMS
TMS
N
NTs
472
f1 (ppm)

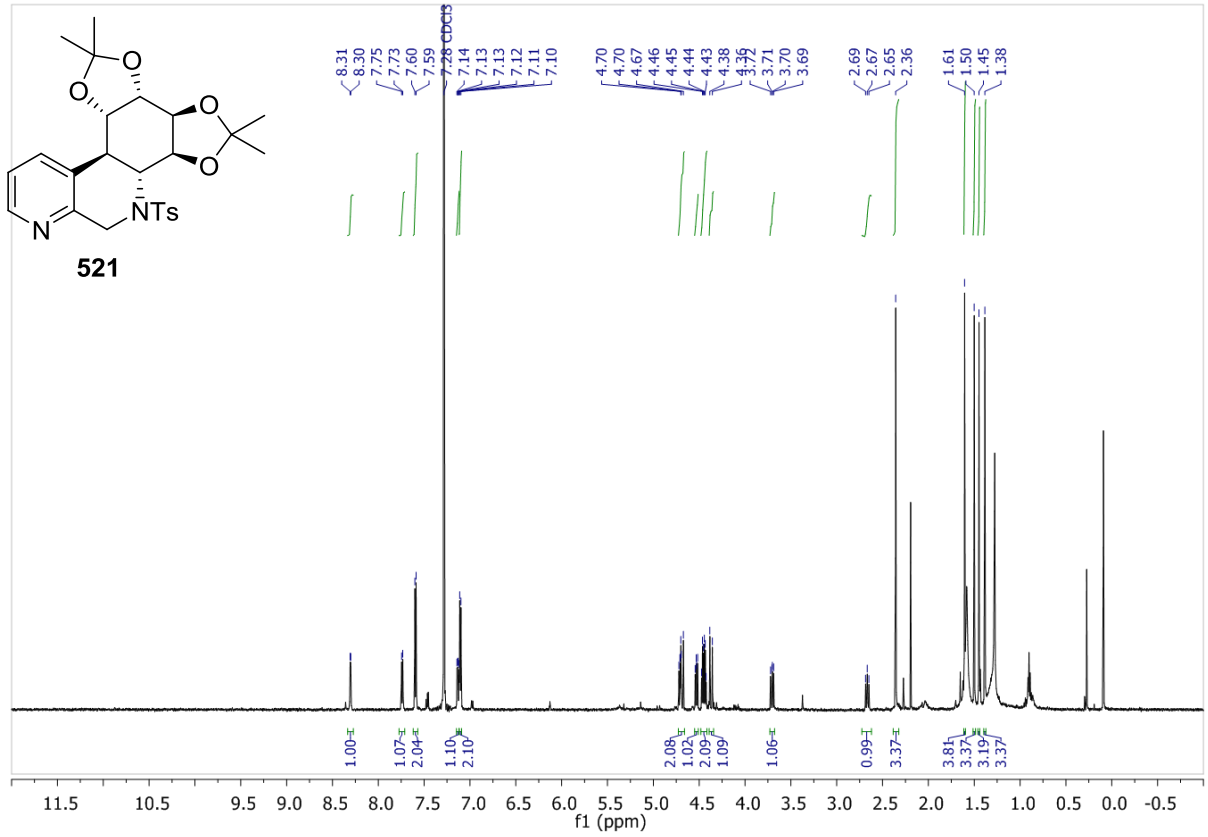

f1 (ppm)

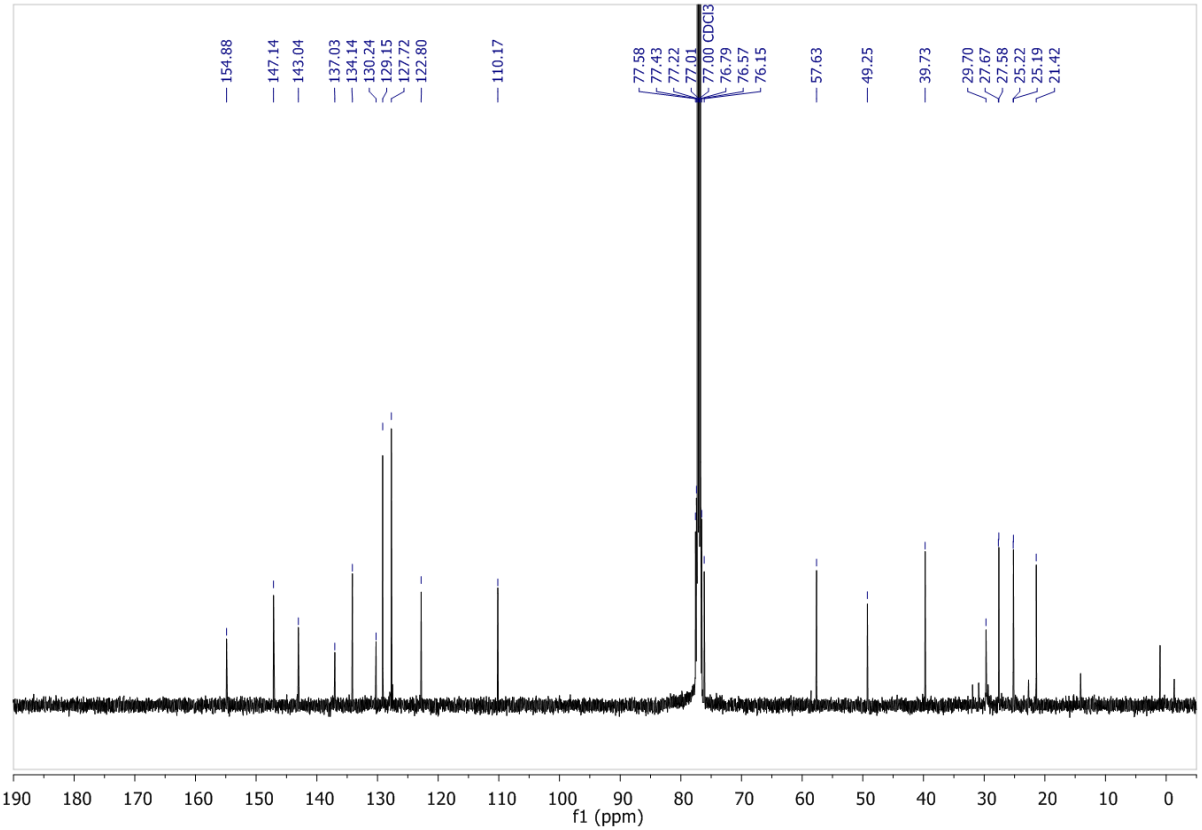

OH
HO
O
O
TMS
TMS
N
NTs
519
7.71
7.48
7.47
7.28
7.00
6.99
5.32
5.04
5.03
5.02
5.01
4.68
4.68
4.40
4.38
4.37
4.13
3.96
3.95
3.95
3.65
3.64
3.63
2.84
2.82
2.82
2.80
2.30
1.56
1.39
0.38
0.33
1.00
1.98
1.93
0.98
1.01
2.01
1.01
1.00
0.97
1.00
3.03
3.09
3.15
8.94
8.98
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

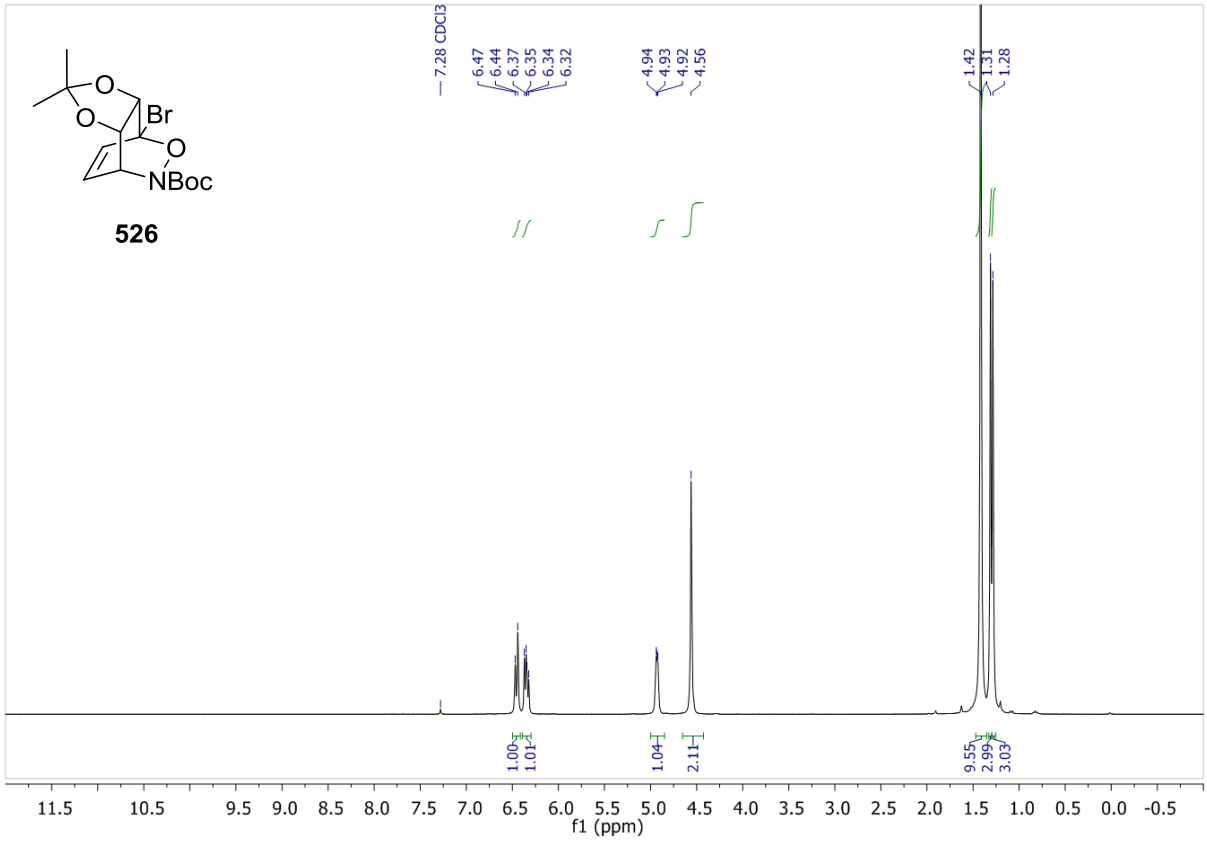

170.74
152.02
142.58
139.69
138.84
137.27
128.93
127.54
110.20
75.89
75.69
70.33
70.03
58.77
51.62
39.97
27.91
25.22
21.47
1.23
1.08
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.71
7.46
7.41
7.28 CDCl3
7.00
6.97
4.86
4.84
4.81
4.79
4.73
4.56
4.50
4.45
4.43
4.41
4.38
4.36
4.35
3.57
3.54
3.53
3.50
2.67
2.64
2.63
2.60
2.30
1.62
1.60
1.49
1.42
1.39
0.37
0.33
TMS
TMS
N
NTs
520
1.00
1.97
1.97
1.01
0.98
0.99
1.11
1.89
0.98
0.99
2.97
0.95
2.98
2.93
2.95
2.92
8.76
8.78
f1 (ppm)

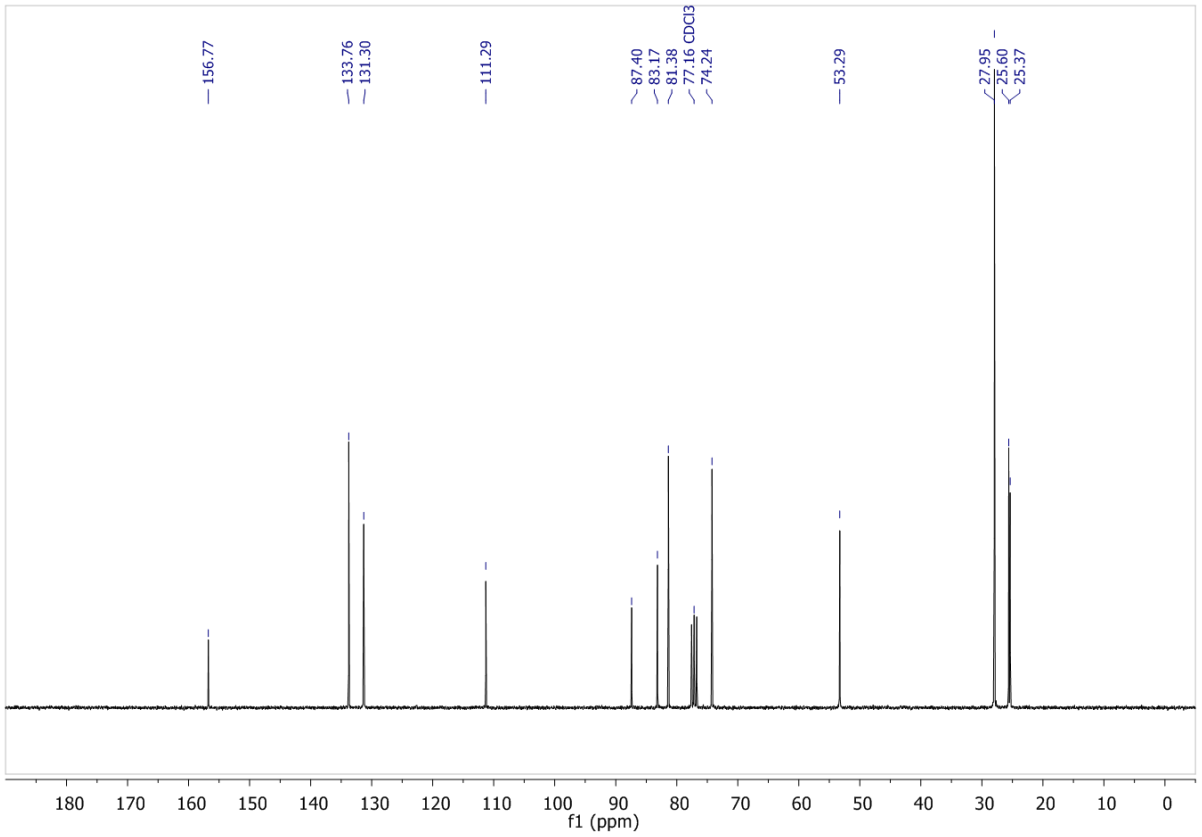

170.79
152.35
142.49
140.01
138.52
136.95
128.92
127.59
127.00
110.15
110.09
77.65
77.22
76.35
76.23
58.28
50.31
39.14
27.70
27.65
25.18
25.13
21.46
1.14
1.04
f1 (ppm)

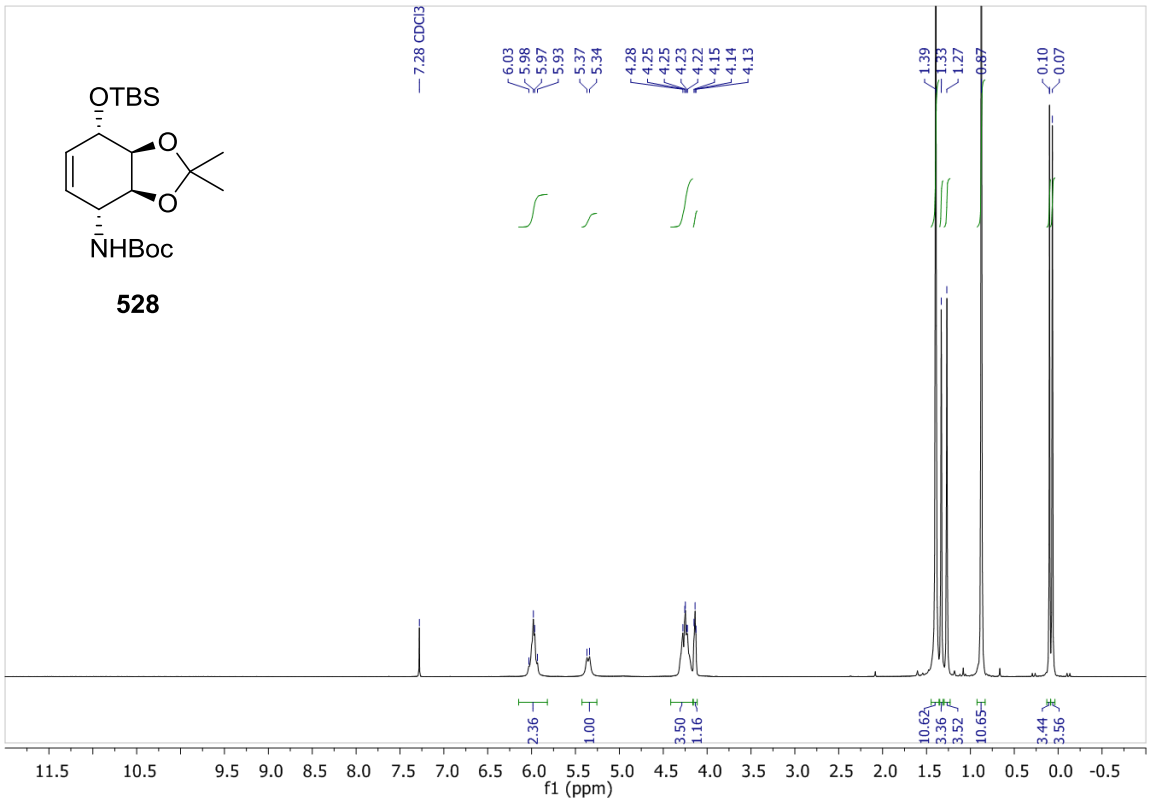

521
8.31
8.30
7.75
7.73
7.60
7.59
7.28 CDCl3
7.14
7.13
7.13
7.12
7.11
7.10
4.70
4.70
4.67
4.46
4.45
4.44
4.43
4.38
3.72
3.71
3.70
3.69
2.69
2.67
2.65
2.36
1.61
1.50
1.45
1.38
1.00
1.07
2.04
1.10
2.10
2.08
1.02
2.09
1.09
1.06
0.99
3.37
3.81
3.37
3.19
3.37
f1 (ppm)

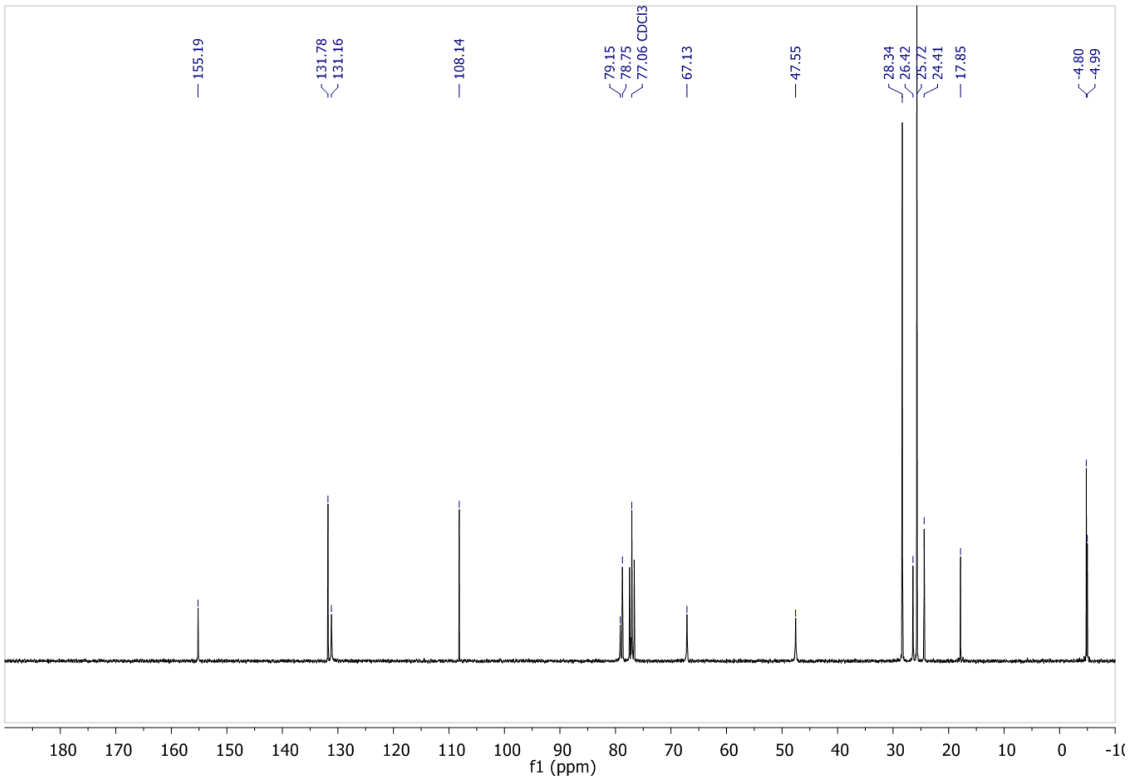

154.88
147.14
143.04
137.03
134.14
130.24
129.15
127.72
122.80
110.17
77.58
77.43
77.22
77.01
77.00 CDCl3
76.79
76.57
76.15
57.63
49.25
39.73
29.70
27.67
27.58
25.22
25.19
21.42
f1 (ppm)

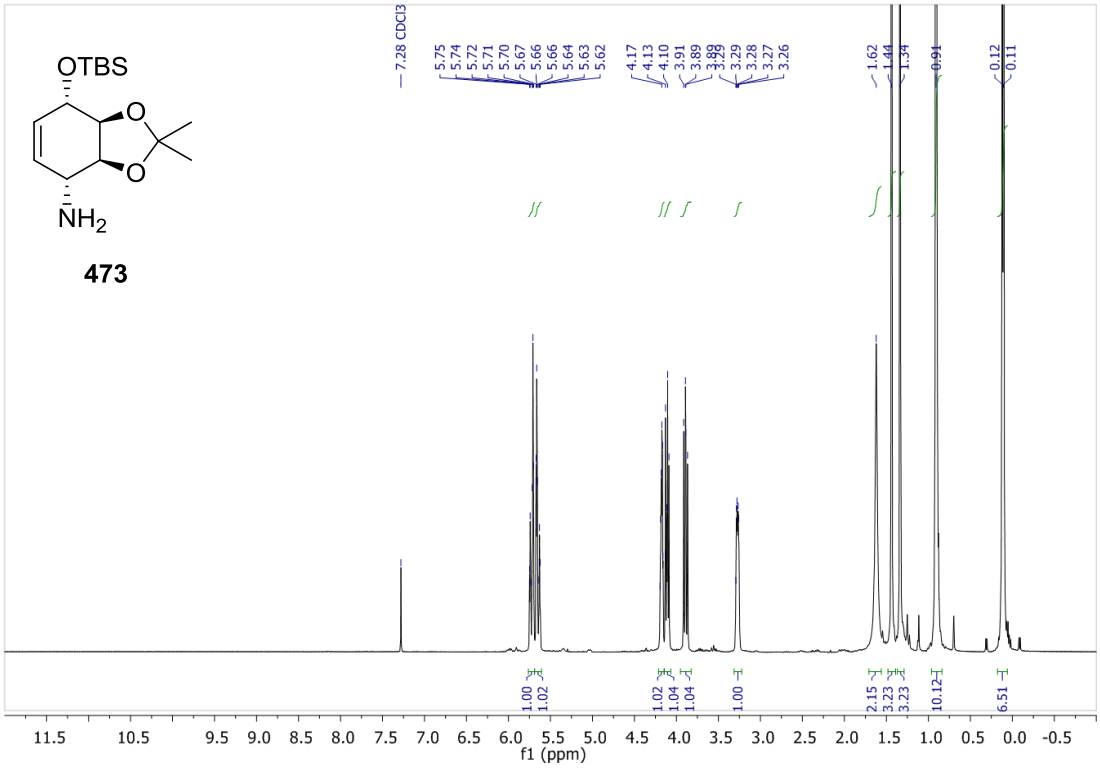

7.28 CDCl3
6.47
6.44
6.37
6.35
6.34
6.32
4.94
4.93
4.92
4.56
1.42
1.31
1.28
1.00
1.01
1.04
2.11
9.55
2.99
3.03
f1 (ppm)

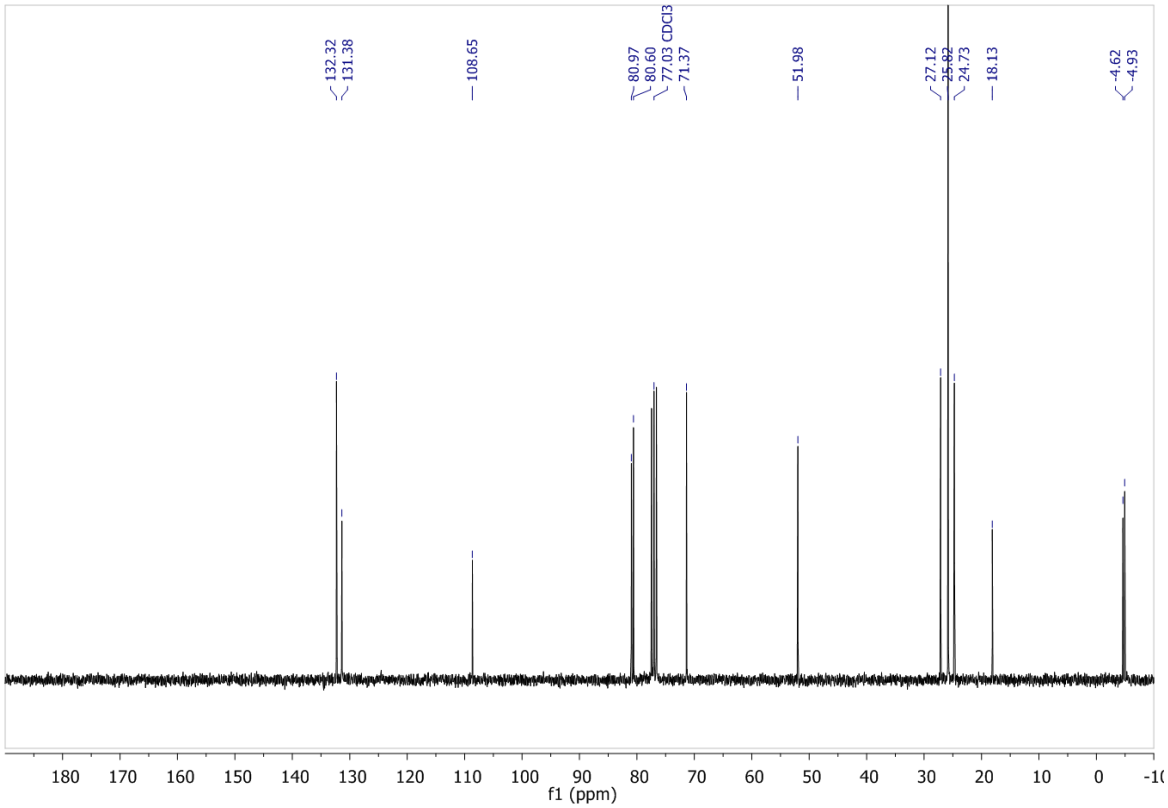
526

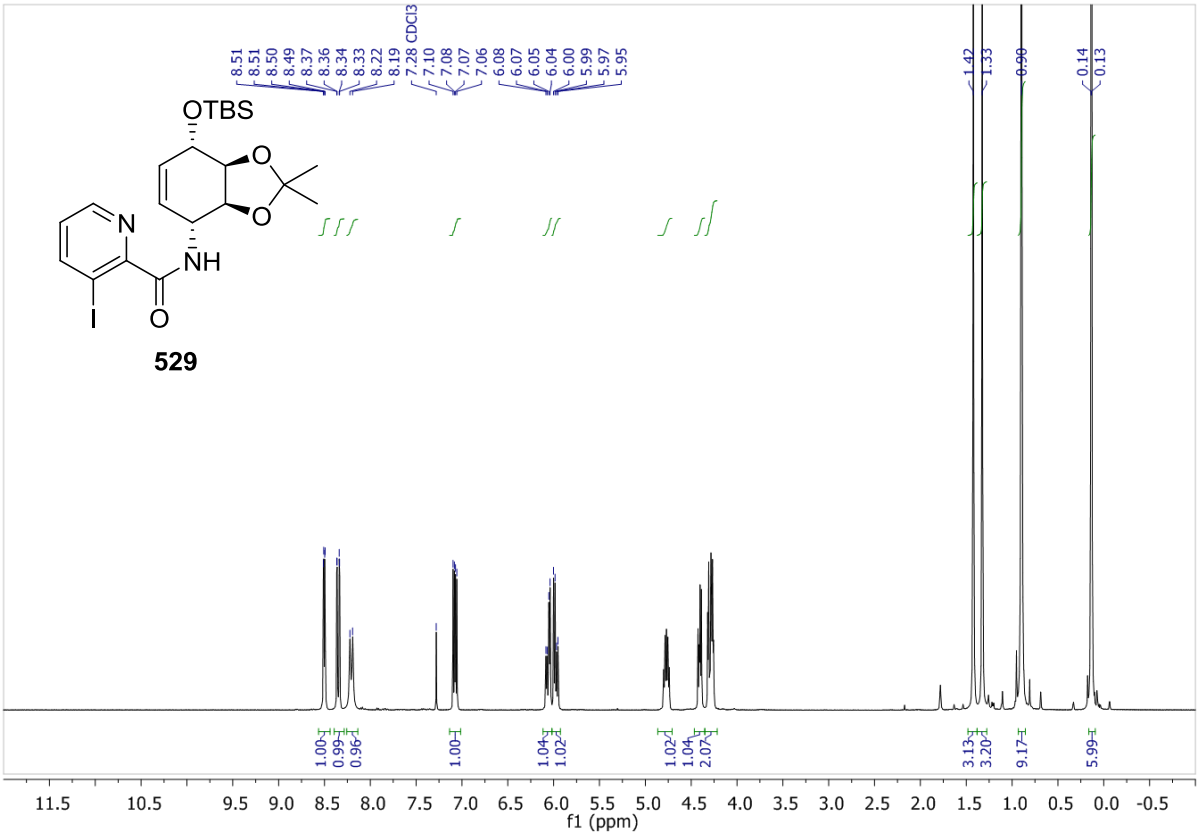

156.77
133.76
131.30
111.29
87.40
83.17
81.38
77.16 CDCl3
74.24
53.29
27.95
25.60
25.37
f1 (ppm)

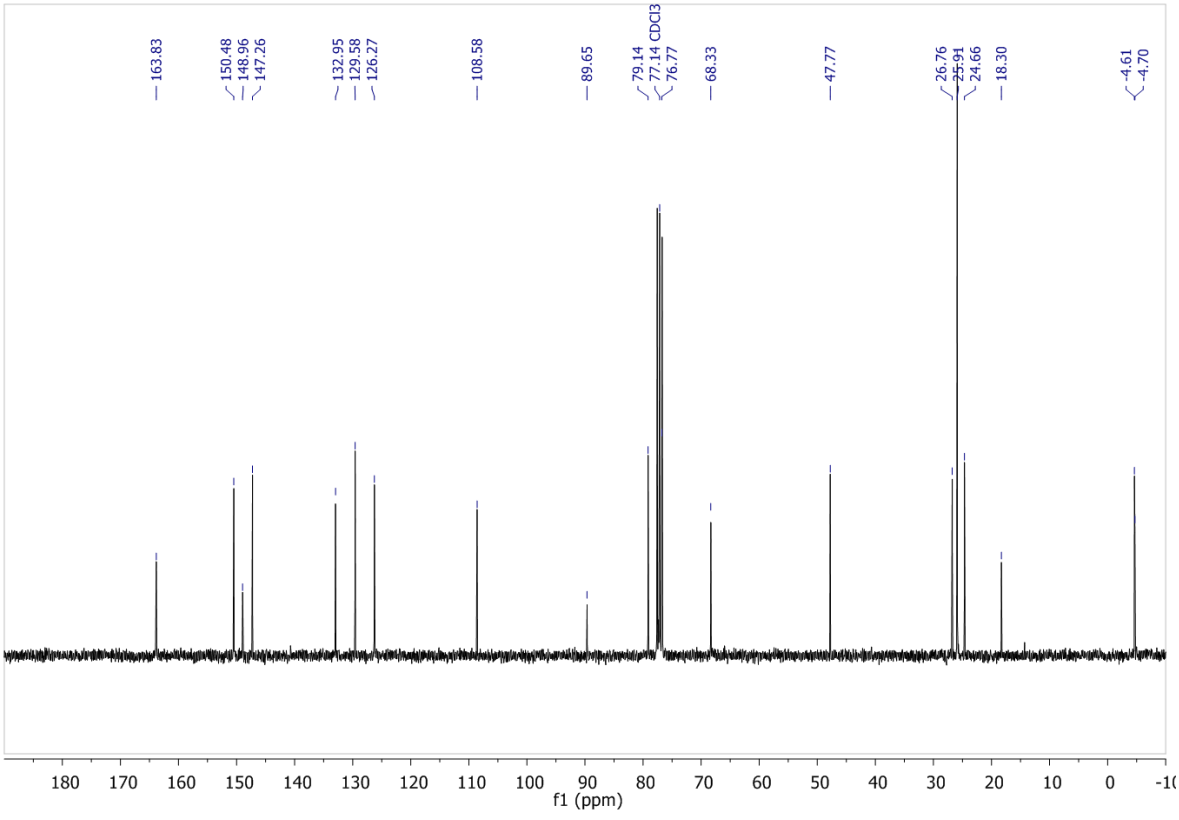

OTBS
O
O
NHBoc
528
7.28 CDCl3
6.03
5.98
5.97
5.93
5.37
5.34
4.28
4.25
4.25
4.23
4.22
4.15
4.14
4.13
1.39
1.33
1.27
0.87
0.10
0.07
2.36
1.00
3.50
1.16
10.62
3.36
3.52
10.65
3.44
3.56
f1 (ppm)

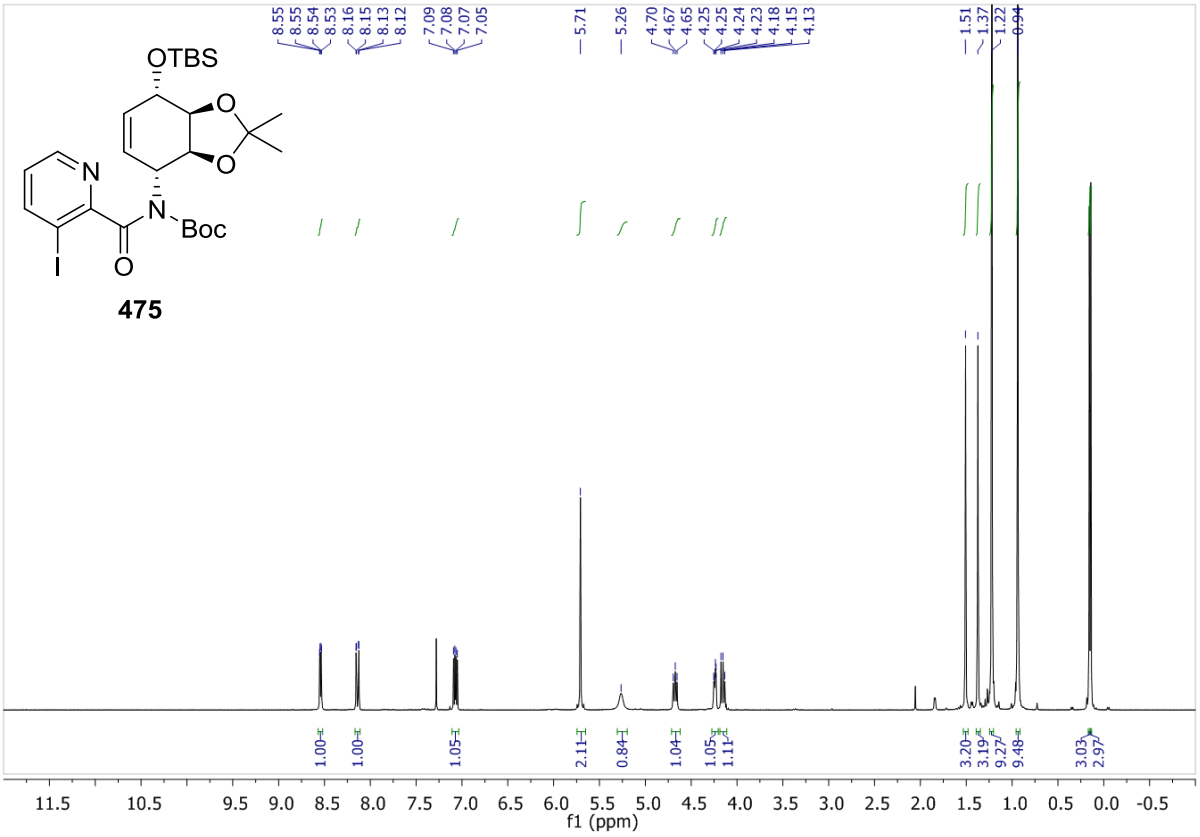

155.19
131.78
131.16
108.14
79.15
78.75
77.06 CDCl3
67.13
47.55
28.34
26.42
25.72
24.41
17.85
-4.80
-4.99
f1 (ppm)

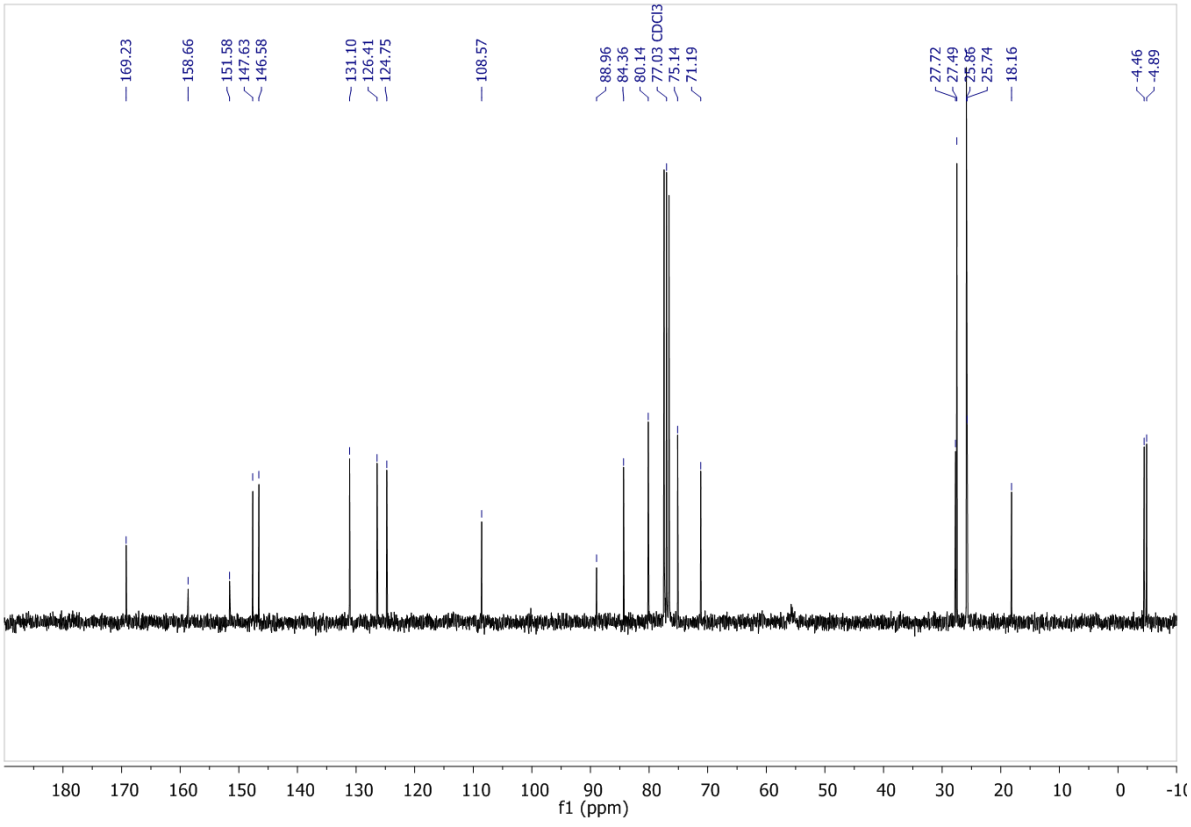

OTBS
O
O
NH2
473
7.28 CDCl3
5.75
5.74
5.72
5.71
5.70
5.67
5.66
5.66
5.64
5.63
5.62
4.17
4.13
4.10
3.91
3.89
3.88
3.29
3.28
3.27
3.26
1.62
1.44
1.34
0.91
0.12
0.11
1.00
1.02
1.02
1.04
1.04
1.00
2.15
3.23
3.23
10.12
6.51
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

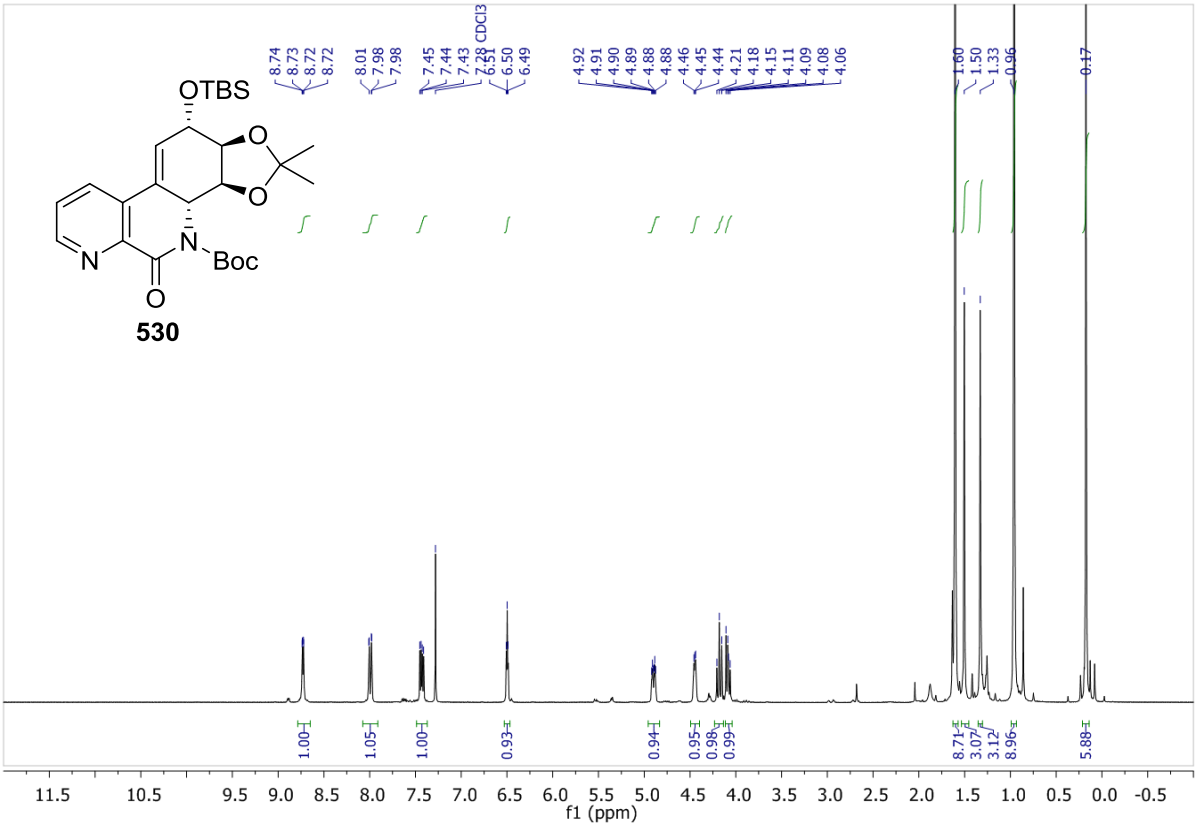

132.32
131.38
108.65
80.97
80.60
77.03 CDCl3
71.37
51.98
27.12
25.82
24.73
18.13
-4.62
-4.93
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

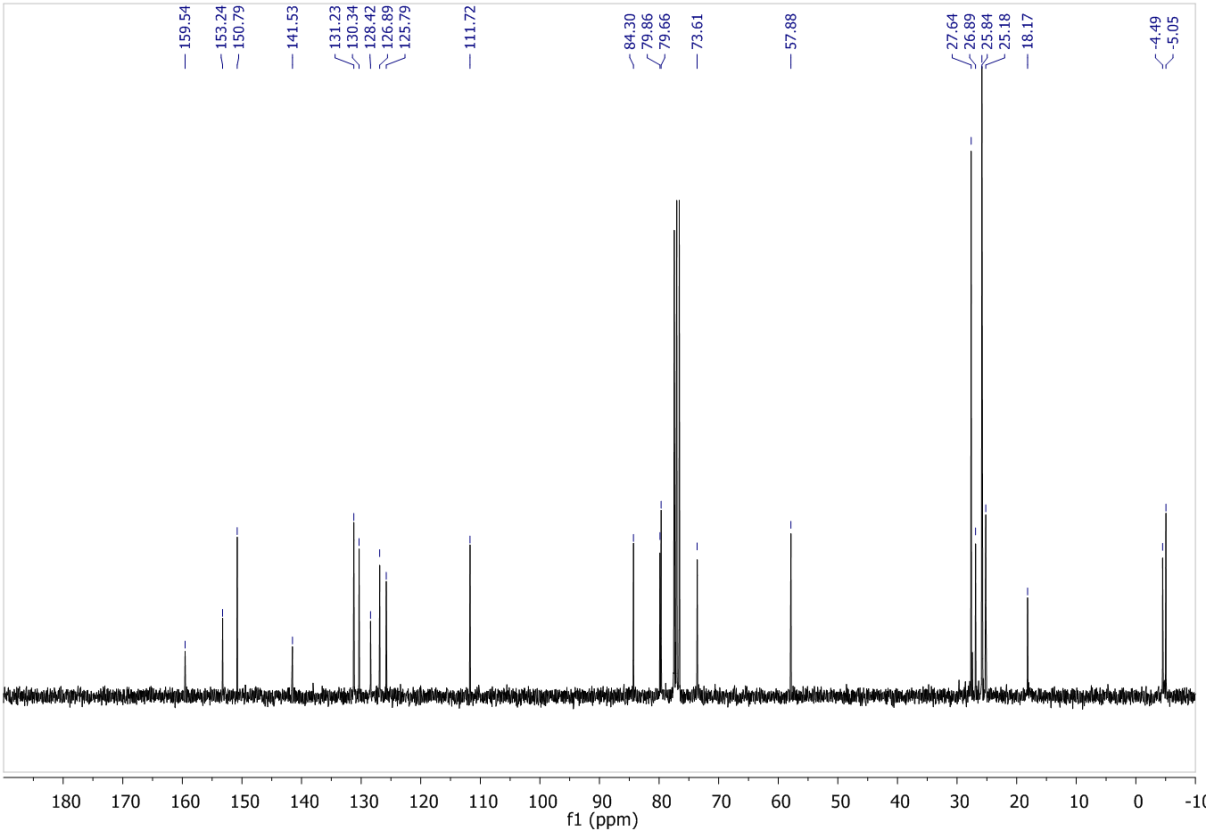

OTBS
O
O
N
NH
I
O
529
8.51
8.51
8.50
8.49
8.37
8.36
8.34
8.33
8.22
8.19
7.28 CDCl3
7.10
7.08
7.07
7.06
6.08
6.07
6.05
6.04
6.00
5.99
5.97
5.95
1.42
1.33
0.00
0.14
0.13
1.00
0.99
0.96
1.00
1.04
1.02
1.02
1.04
2.07
3.13
3.20
9.17
5.99
f1 (ppm)

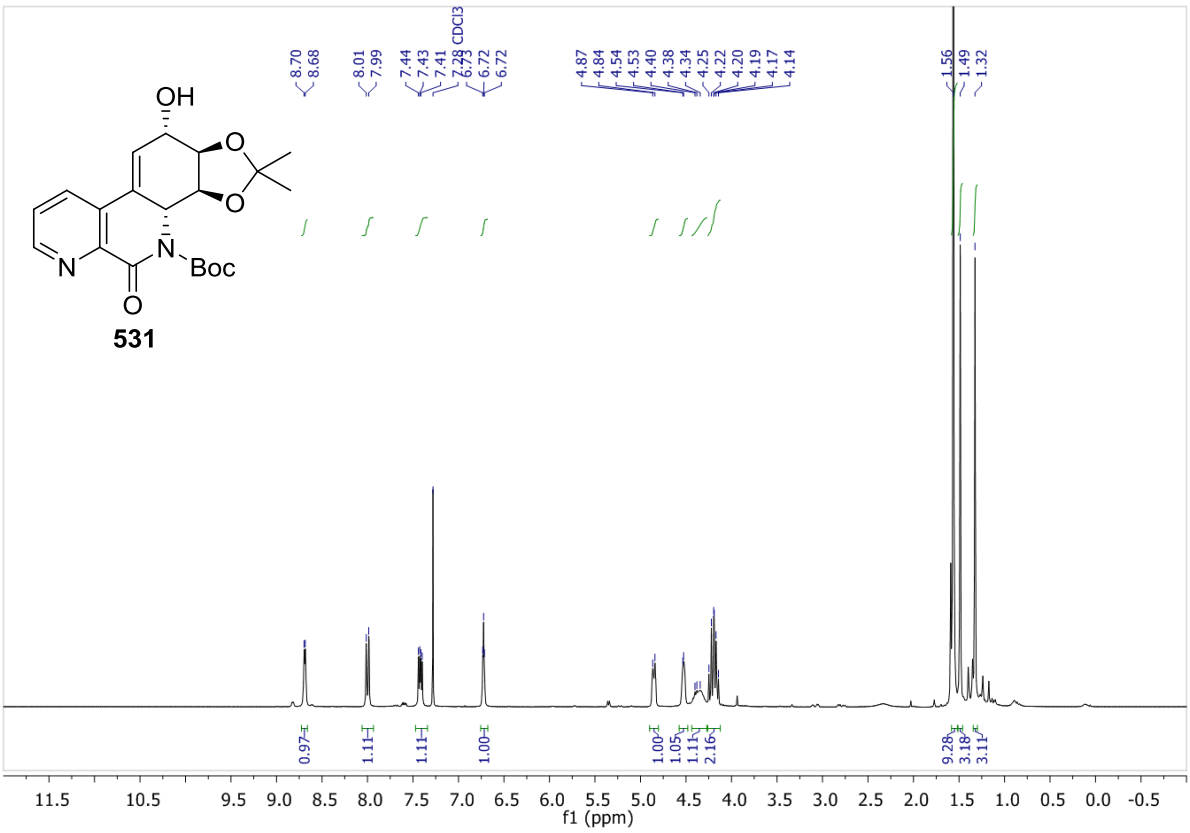

163.83
150.48
148.96
147.26
132.95
129.58
126.27
108.58
89.65
79.14
77.14 CDCl3
76.77
68.33
47.77
26.76
25.91
24.66
18.30
-4.61
-4.70
f1 (ppm)

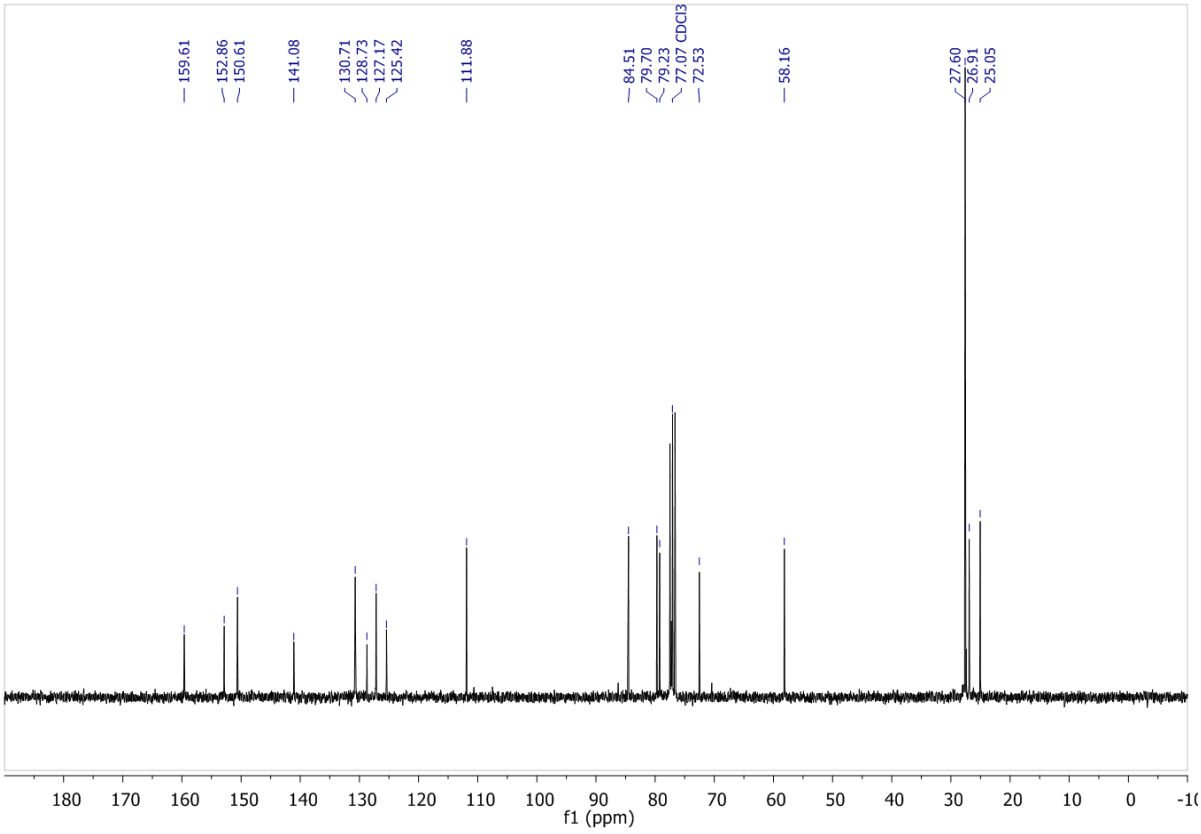

OTBS
O
O
N
N
Boc
I
O
475
8.55
8.55
8.54
8.53
8.16
8.15
8.13
8.12
7.09
7.08
7.07
7.05
5.71
5.26
4.70
4.67
4.65
4.25
4.25
4.24
4.23
4.18
4.15
4.13
1.51
1.37
1.22
0.94
1.00
1.00
1.05
2.11
0.84
1.04
1.05
1.11
3.20
3.19
9.27
9.48
3.03
2.97
f1 (ppm)

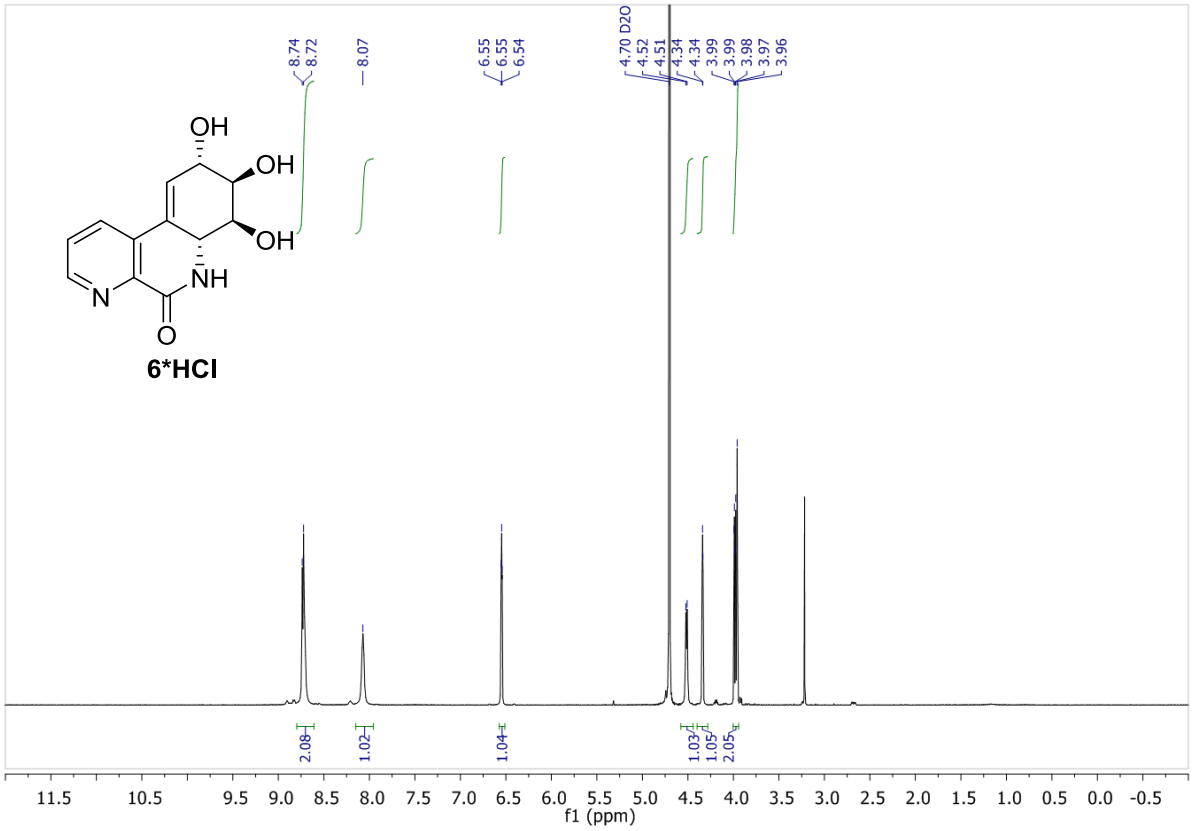

169.23
158.66
151.58
147.63
146.58
131.10
126.41
124.75
108.57
88.96
84.36
80.14
77.03 CDCl3
75.14
71.19
27.72
27.49
25.86
25.74
18.16
-4.46
-4.89
f1 (ppm)

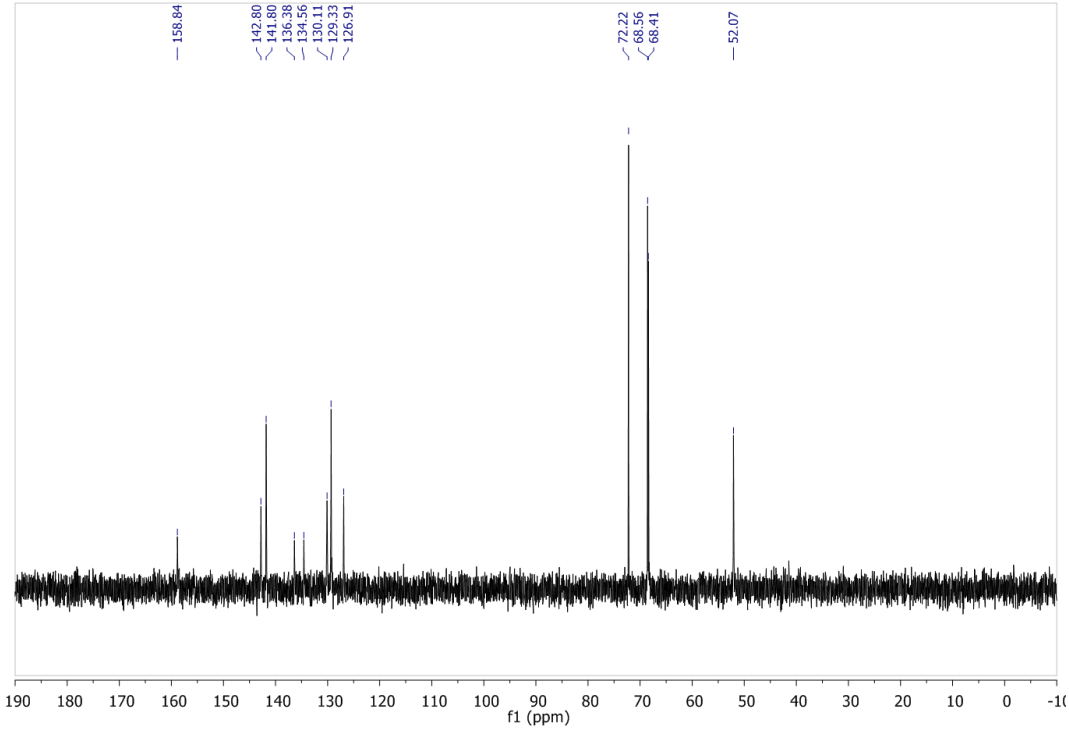

OTBS
O
O
N
N
Boc
O
530
8.74
8.73
8.72
8.72
8.01
7.98
7.98
7.45
7.44
7.43
7.28 CDCl3
6.51
6.50
6.49
4.92
4.91
4.90
4.89
4.88
4.88
4.46
4.45
4.44
4.21
4.18
4.15
4.11
4.09
4.08
4.06
1.60
1.50
1.33
0.96
0.17
1.00
1.05
1.00
0.93
0.94
0.95
0.98
0.99
8.71
3.07
3.12
8.96
5.88
f1 (ppm)

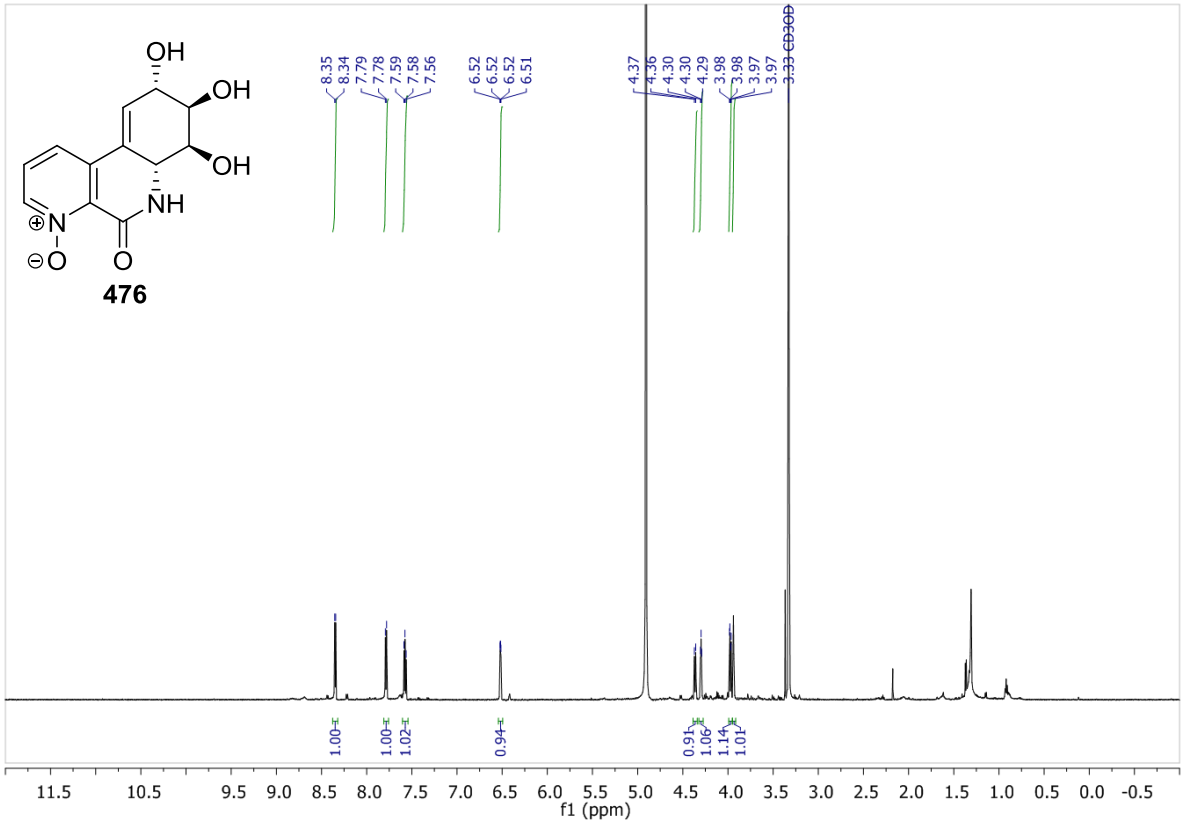

159.54
153.24
150.79
141.53
131.23
130.34
128.42
126.89
125.79
111.72
84.30
79.86
79.66
73.61
57.88
27.64
26.89
25.84
25.18
18.17
-4.49
-5.05
f1 (ppm)

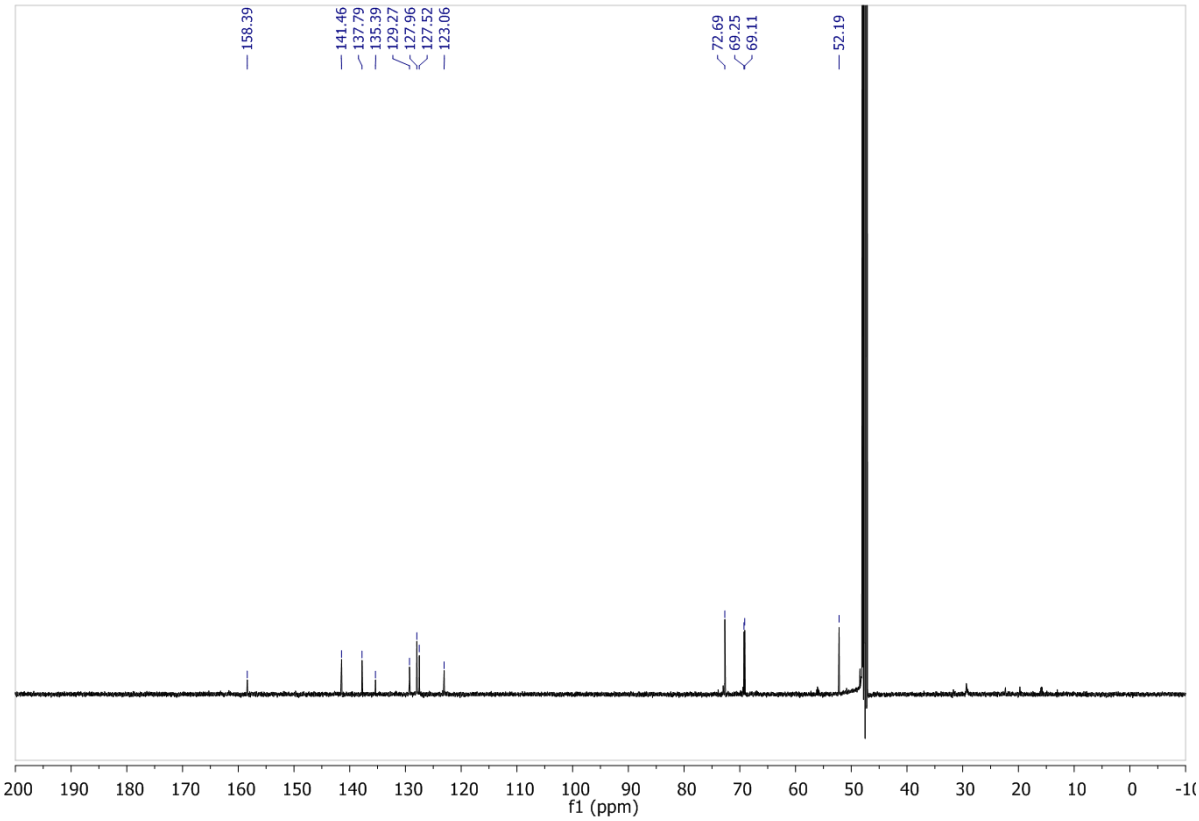

OH
O
O
N
N
Boc
O
531
8.70
8.68
8.01
7.99
7.44
7.43
7.41
7.28 CDCl3
6.73
6.72
6.72
4.87
4.84
4.54
4.53
4.40
4.38
4.34
4.25
4.22
4.20
4.19
4.17
4.14
1.56
1.49
1.32
0.97
1.11
1.11
1.00
1.00
1.05
1.11
2.16
9.28
3.18
3.11
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

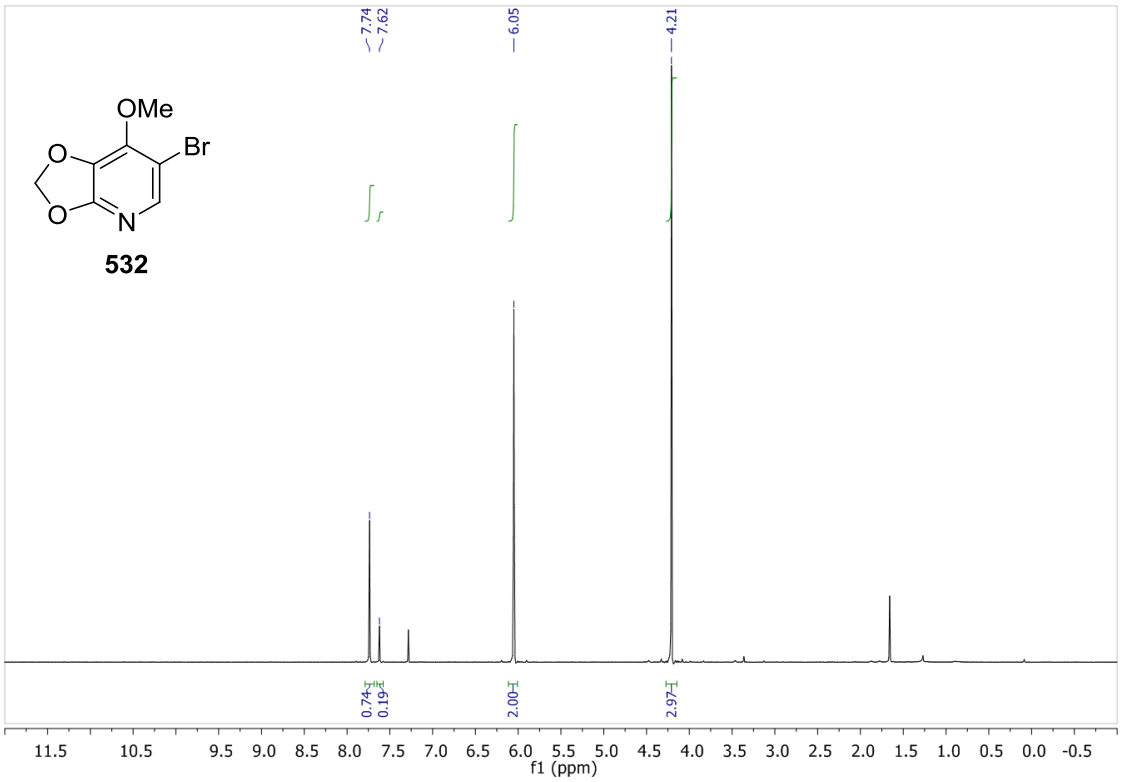

159.61
152.86
150.61
141.08
130.71
128.73
127.17
125.42
111.88
84.51
79.70
79.23
77.07 CDCl3
72.53
58.16
27.60
26.91
25.05
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

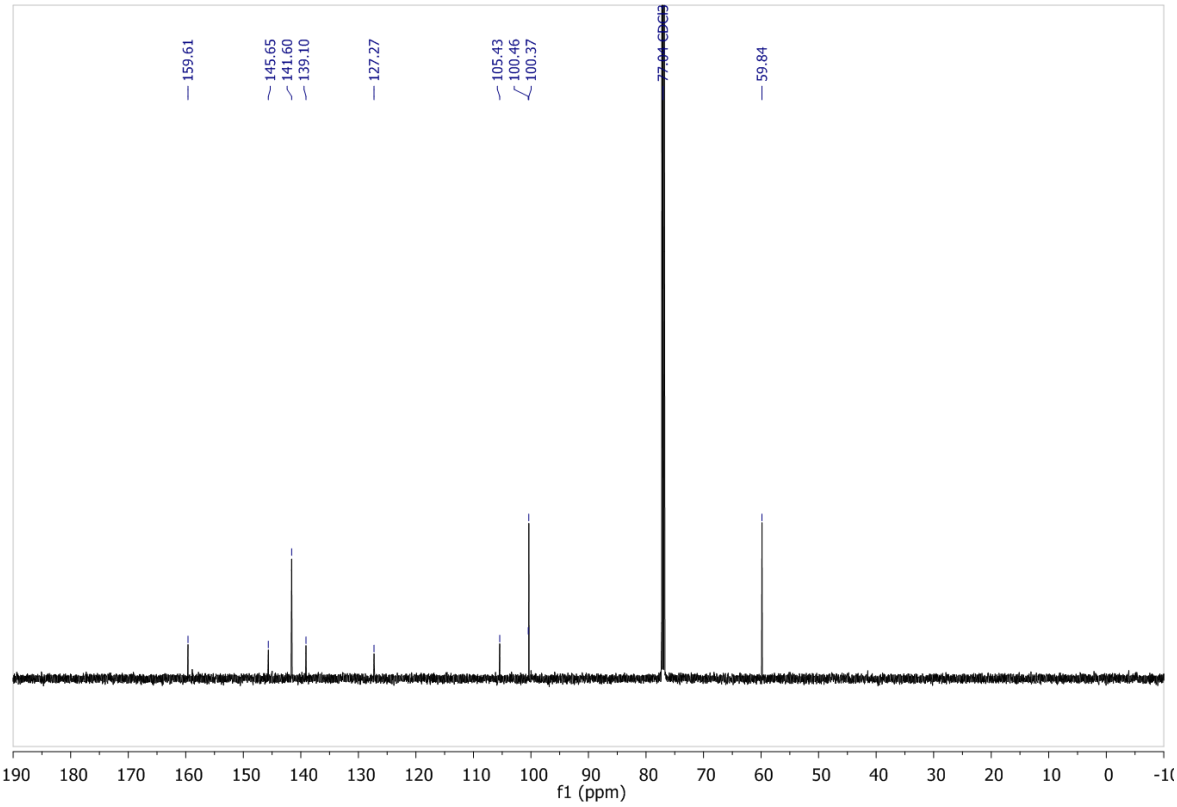

8.74
8.72
8.07
6.55
6.55
6.54
4.70 D2O
4.52
4.51
4.34
4.34
3.99
3.99
3.98
3.97
3.96
OH
OH
OH
NH
N
O
6*HCl
2.08
1.02
1.04
1.03
1.05
2.05
f1 (ppm)

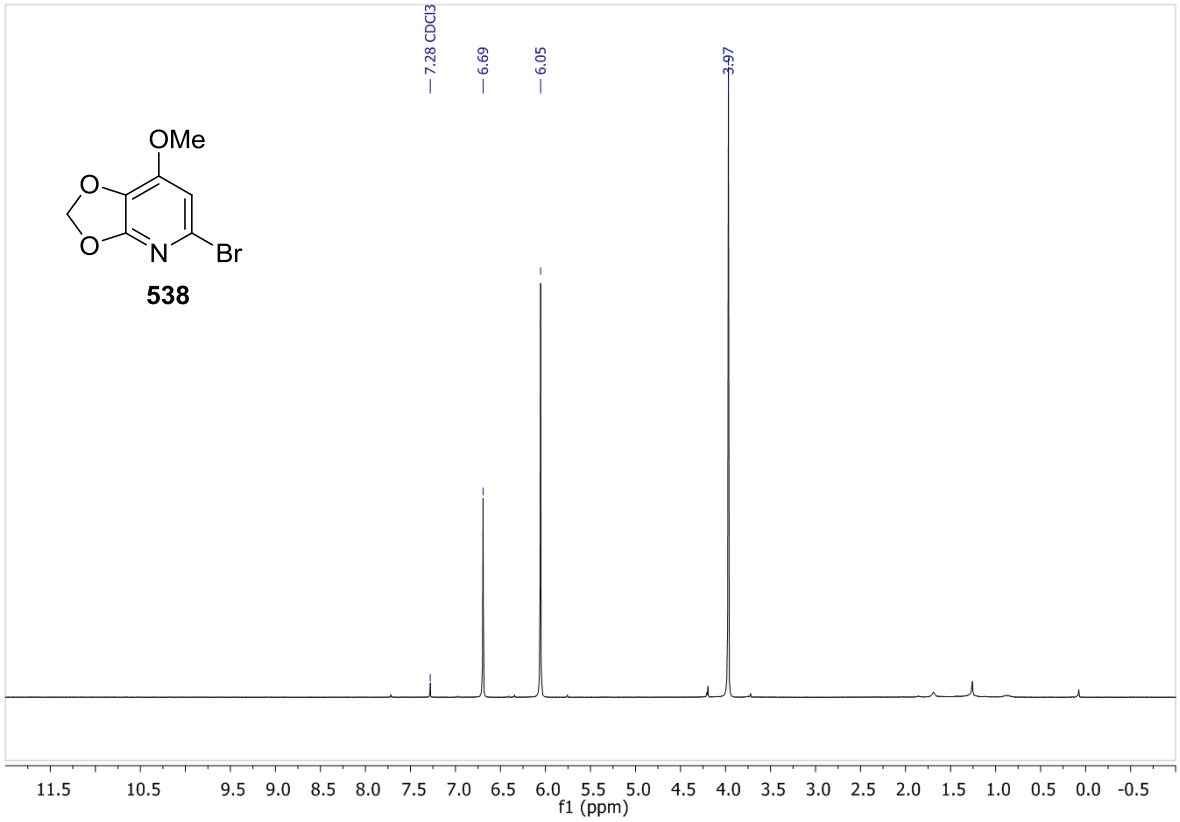

158.84
142.80
141.80
136.38
134.56
130.11
129.33
126.91
72.22
68.56
68.41
52.07
f1 (ppm)

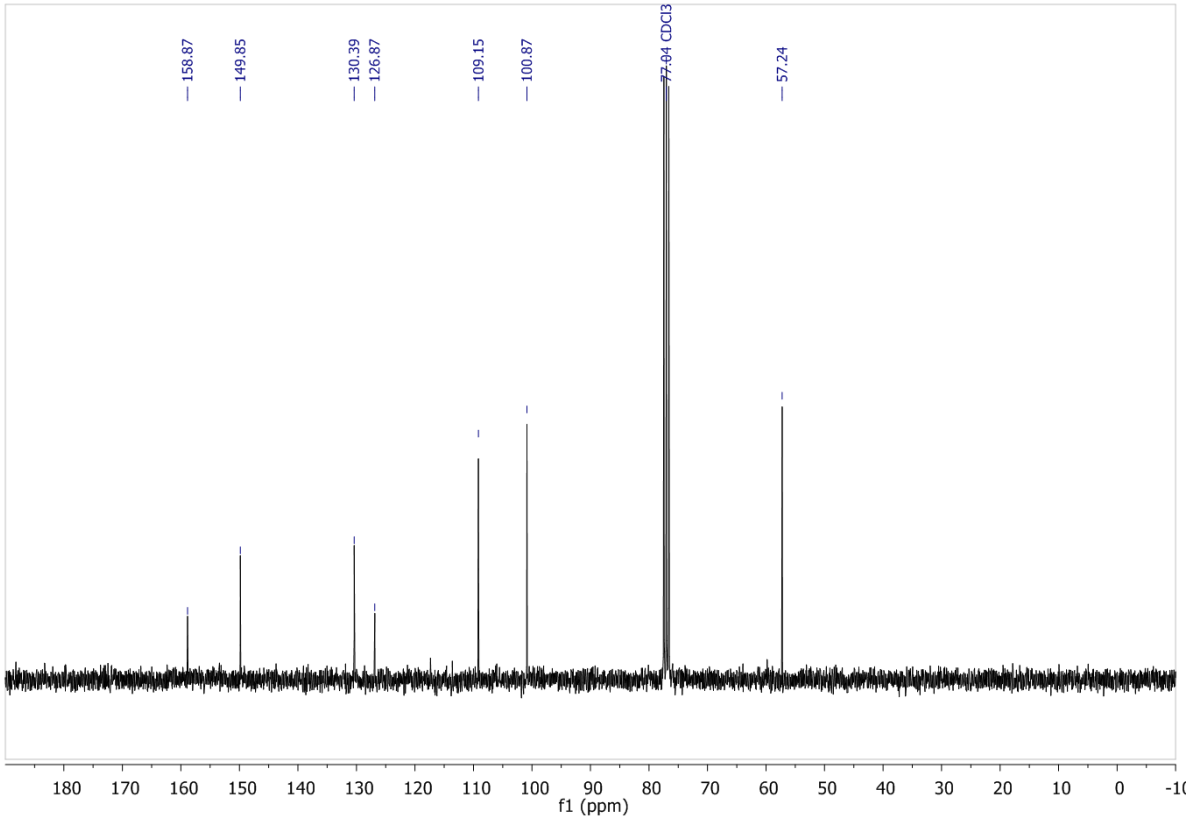

OH
OH
OH
NH
O
O
476
8.35
8.34
7.79
7.78
7.59
7.58
7.56
6.52
6.52
6.52
6.51
4.37
4.36
4.30
4.30
4.29
3.98
3.98
3.97
3.97
3.33
1.00
1.00
1.02
0.94
0.91
1.06
1.14
1.01
f1 (ppm)

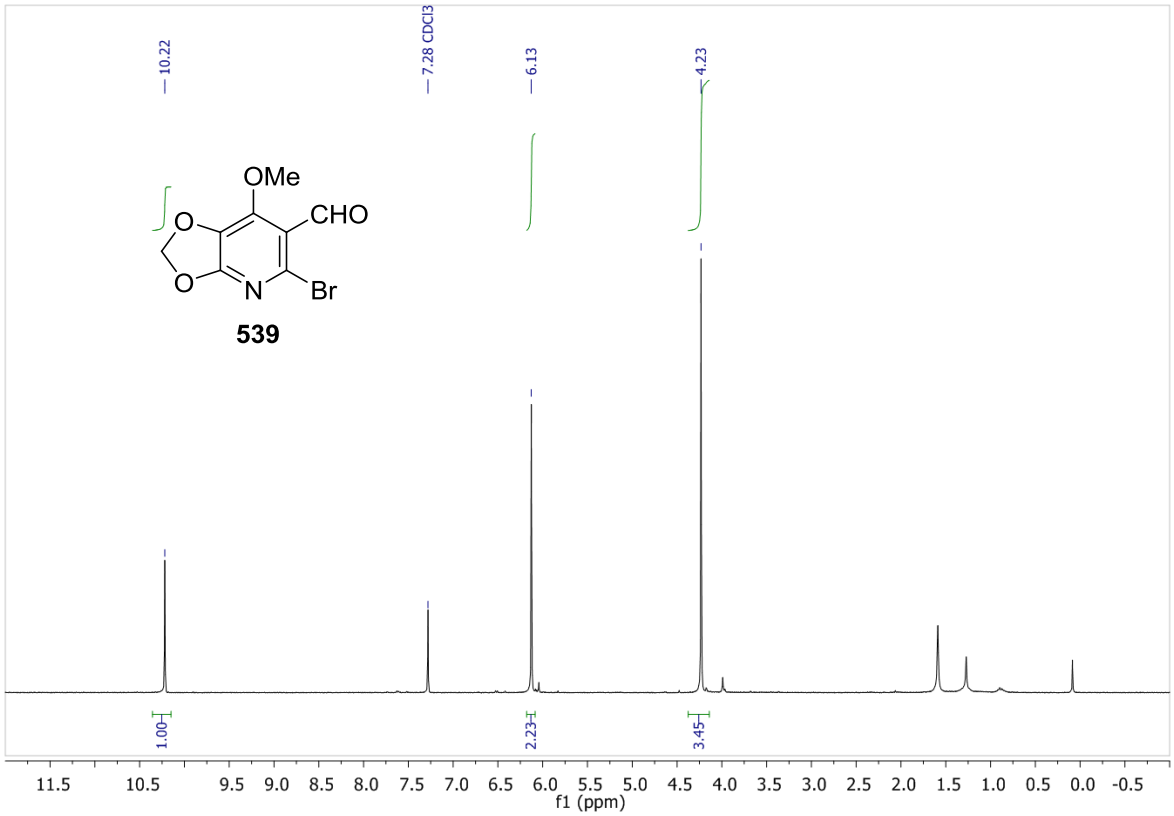

158.39
141.46
137.79
135.39
129.27
127.96
127.52
123.06
72.69
69.25
69.11
52.19
f1 (ppm)

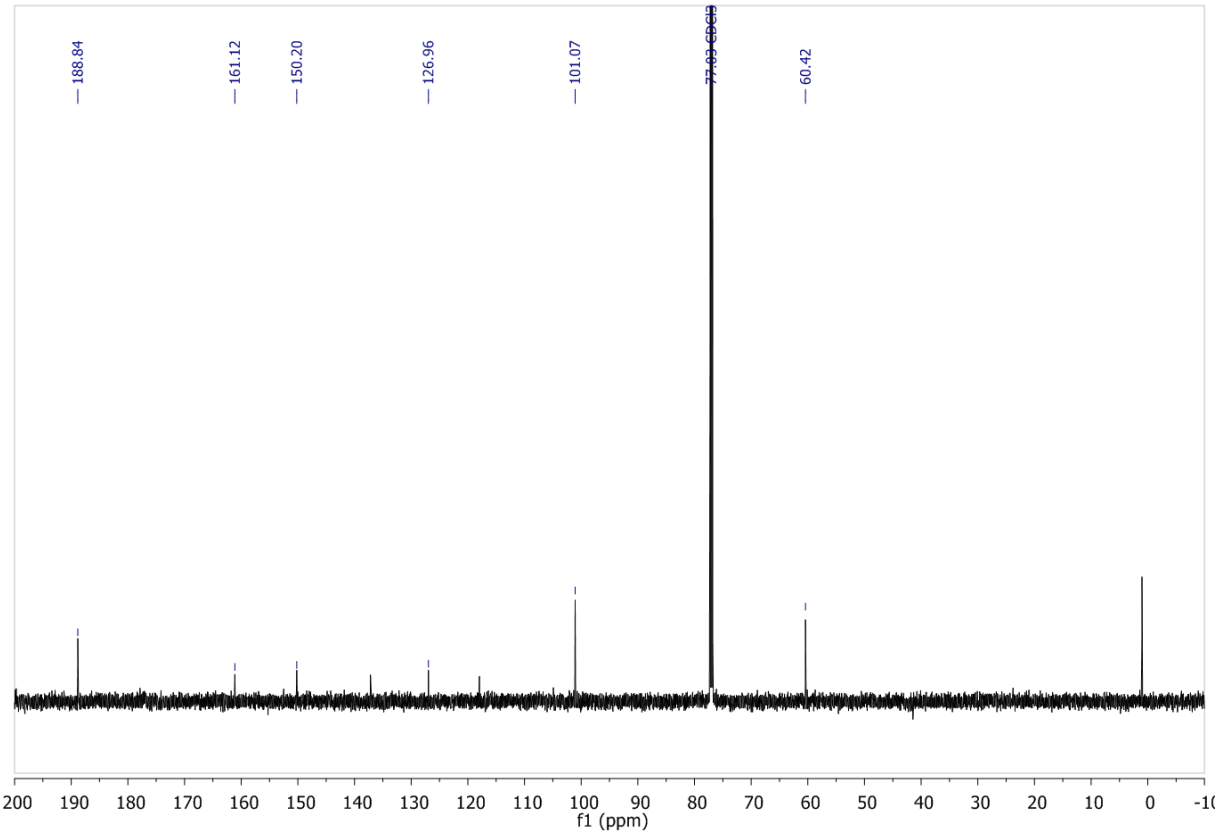

OMe
Br
O
O
N
532
7.74
7.62
6.05
4.21
0.74
0.19
2.00
2.97
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

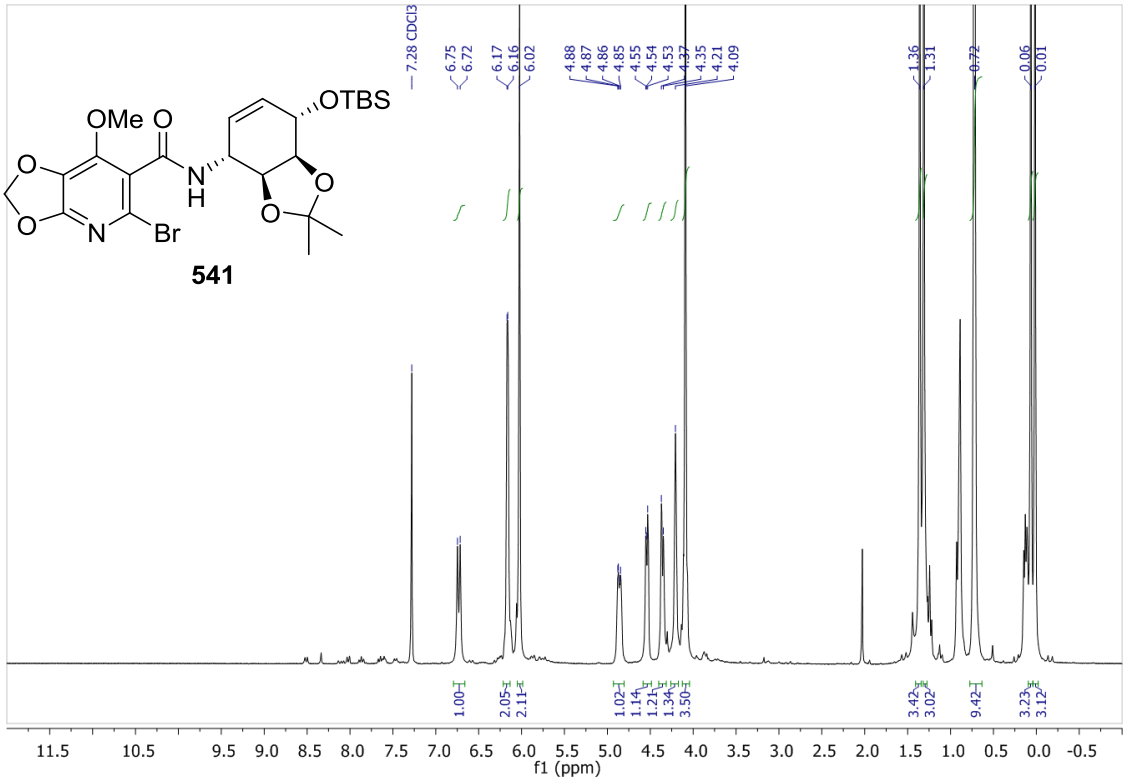

159.61
145.65
141.60
139.10
127.27
105.43
100.46
100.37
77.04
59.84
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

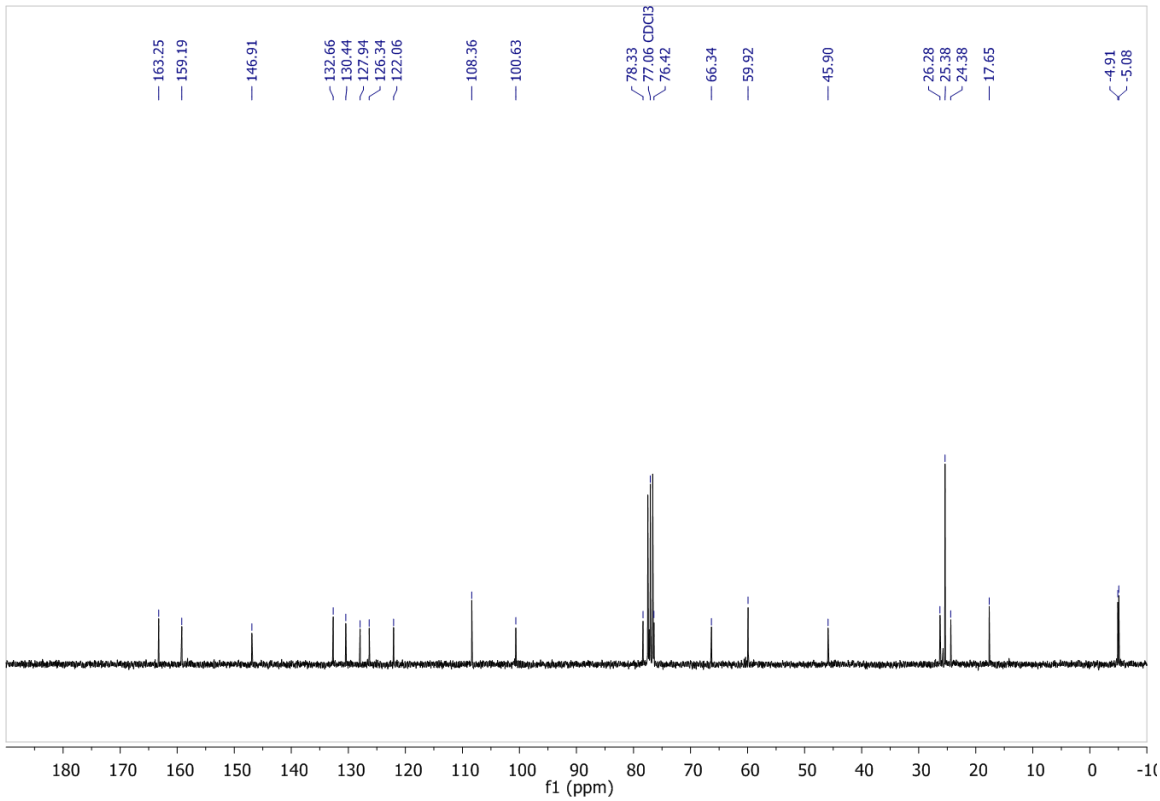

7.28 CDCl3
6.69
6.05
3.97
OMe
O
O
N
Br
538
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

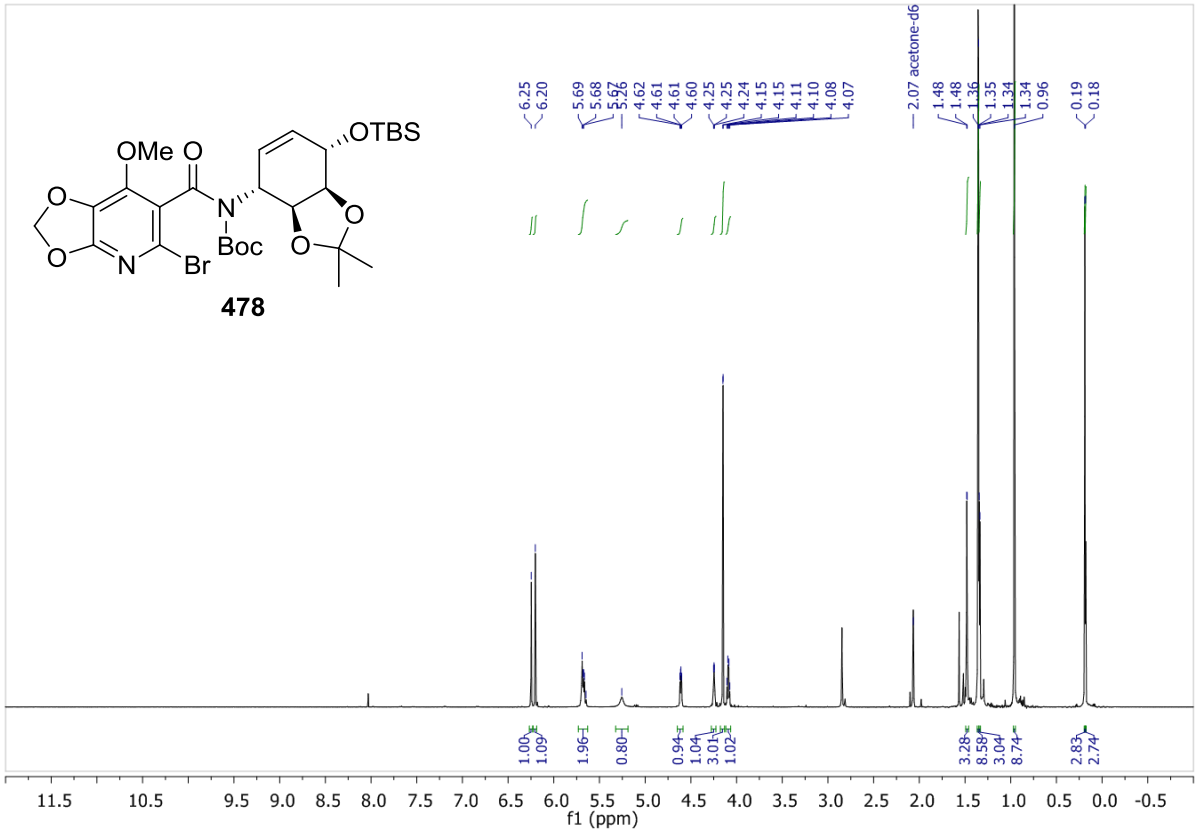

158.87
149.85
130.39
126.87
109.15
100.87
77.04 CDCl3
57.24
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

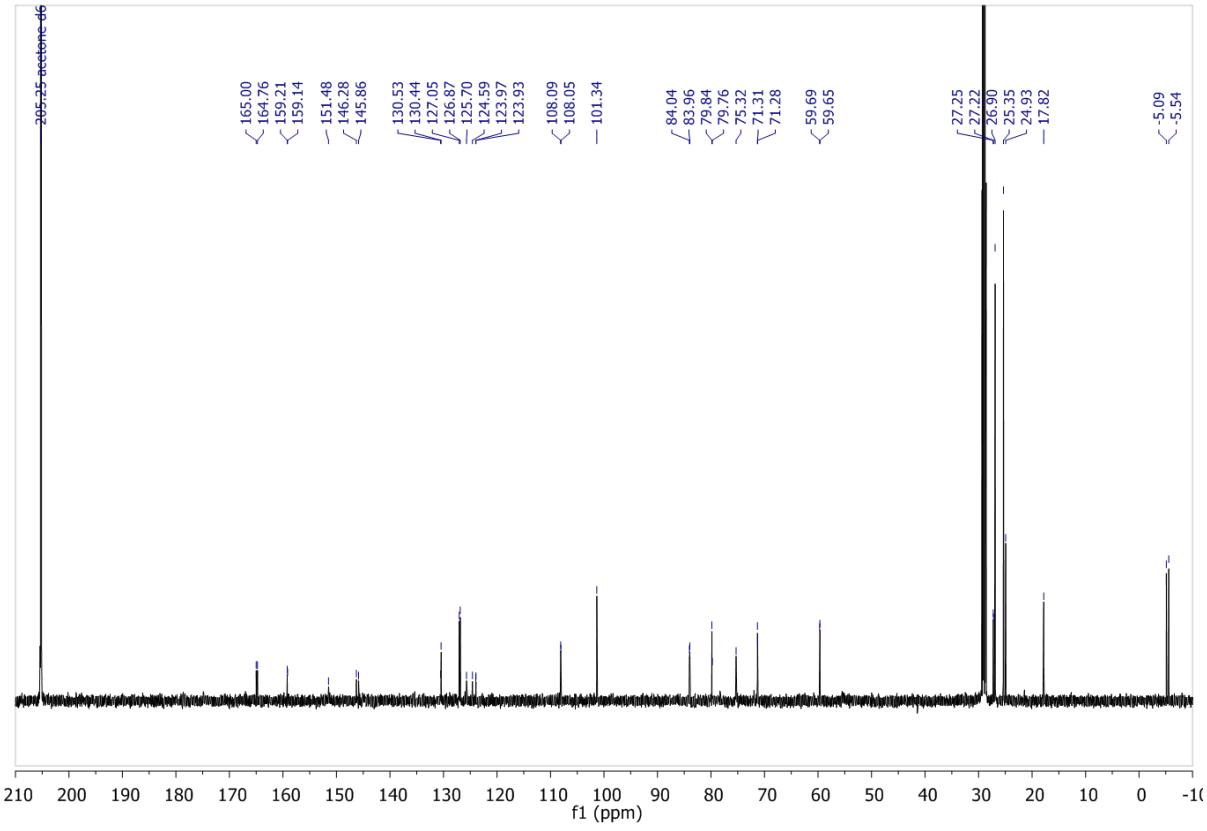

10.22
7.28 CDCl3
6.13
4.23
OMe
CHO
O
O
N
Br
539
1.00
2.23
3.45
11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

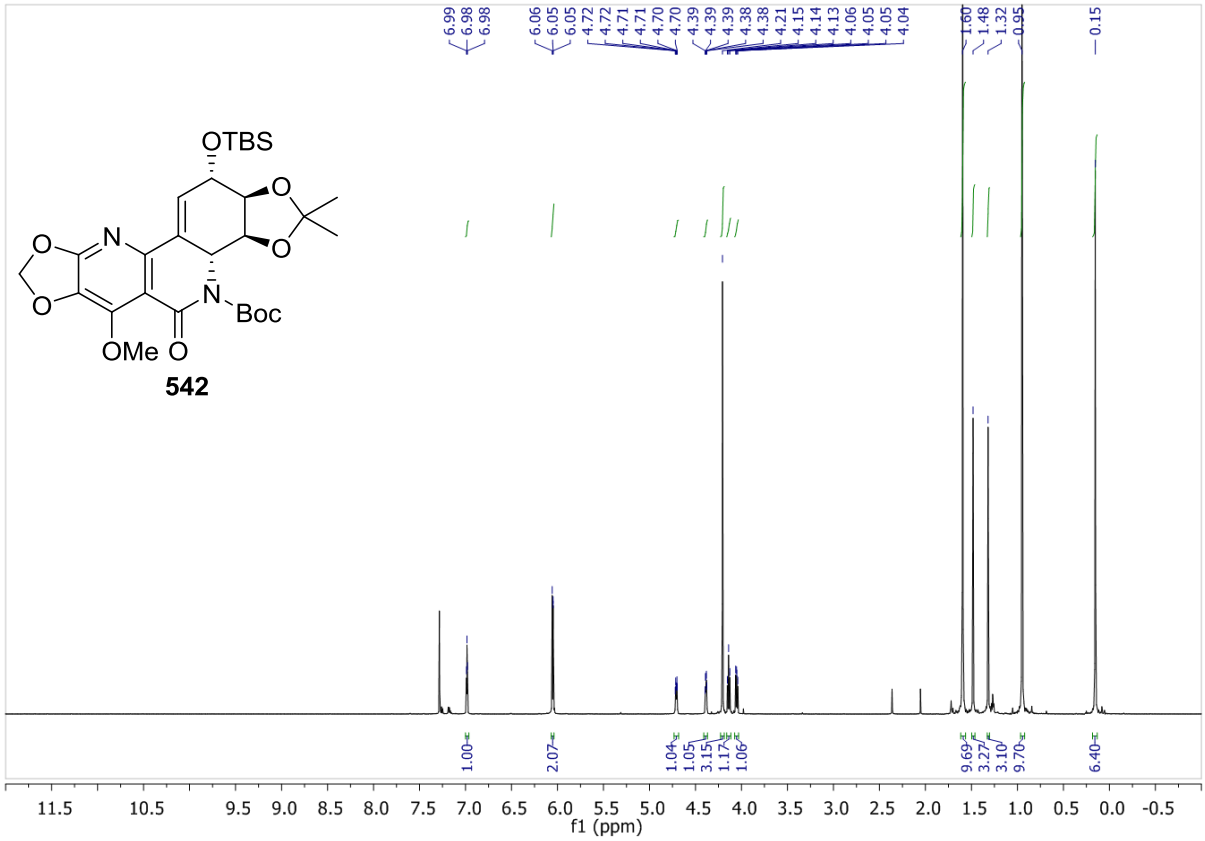

188.84
161.12
150.20
126.96
101.07
77.03 CDCl3
60.42
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

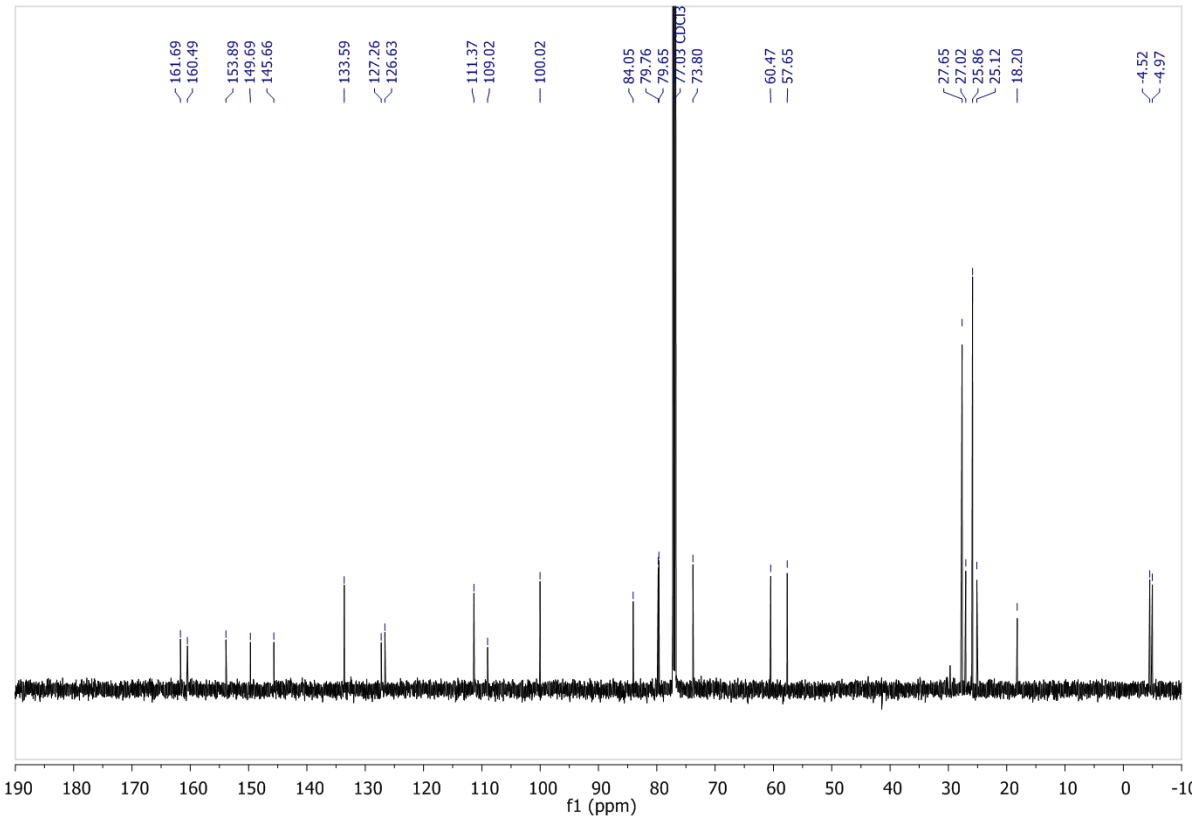

OMe O
OTBS
O
O
N
H
N
Br
O
O
541
7.28 CDCl3
6.75
6.72
6.17
6.16
6.02
4.88
4.87
4.86
4.85
4.55
4.54
4.53
4.37
4.35
4.21
4.09
1.36
1.31
0.72
0.06
0.01
1.00
2.05
2.11
1.02
1.14
1.21
1.34
3.50
3.42
3.02
9.42
3.23
3.12
11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

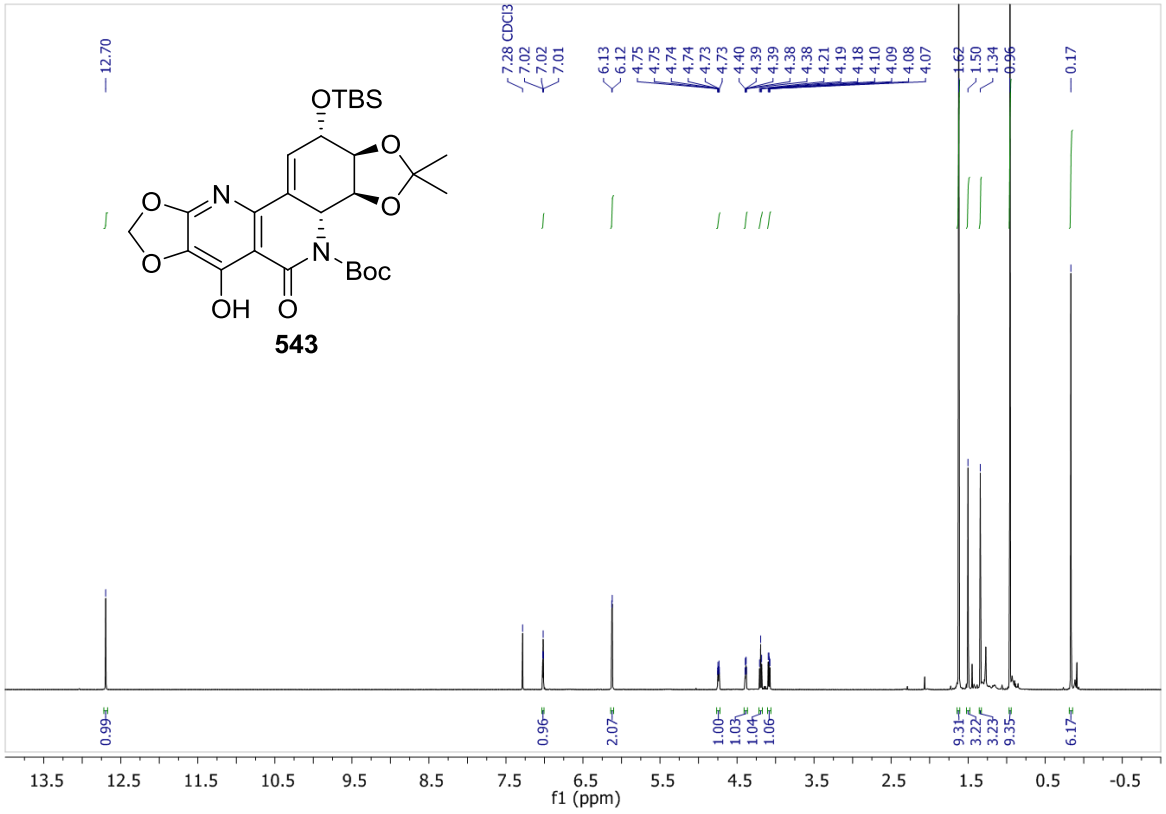

163.25
159.19
146.91
132.66
130.44
127.94
126.34
122.06
108.36
100.63
78.33
77.06 CDCl3
76.42
66.34
59.92
45.90
26.28
25.38
24.38
17.65
-4.91
-5.08
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

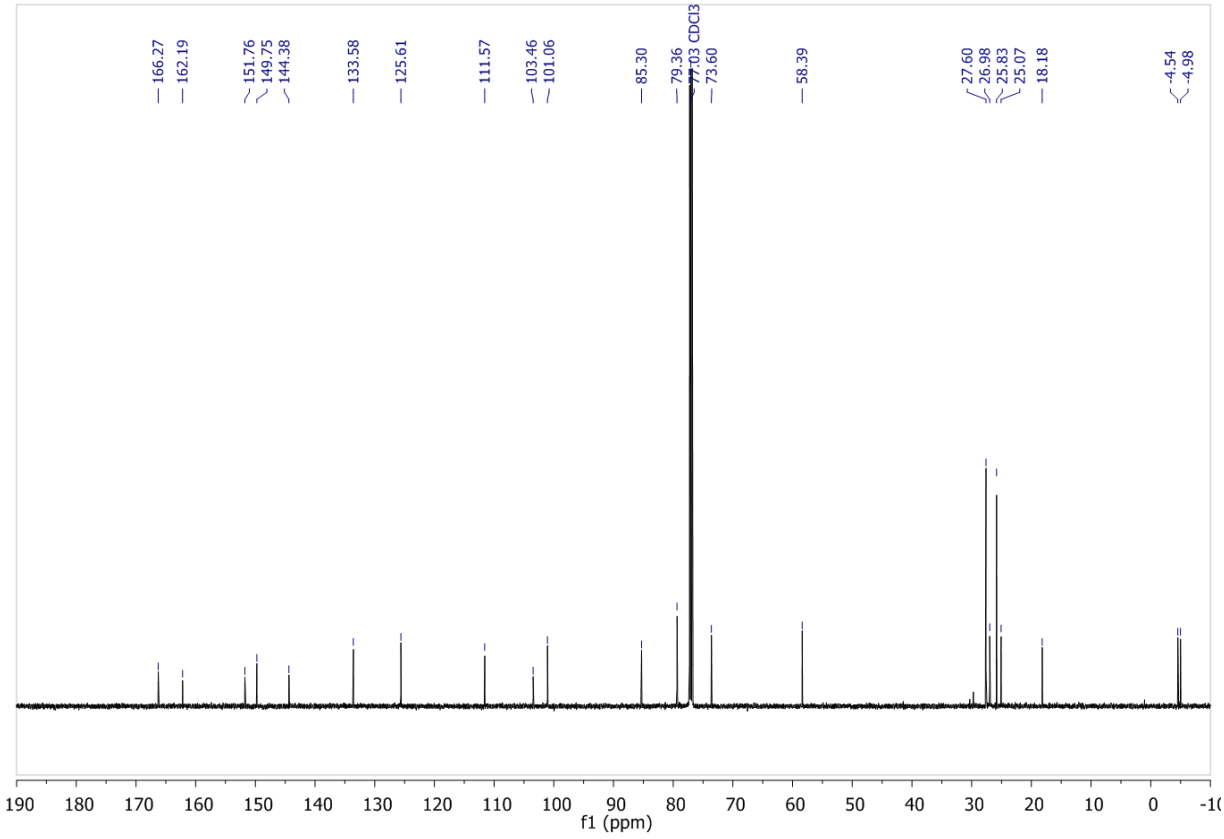

OMe O
OTBS
O
O
N
Br
Boc
O
O
N
O
478
6.25
6.20
5.69
5.68
5.67
5.26
4.62
4.61
4.61
4.60
4.25
4.25
4.24
4.15
4.15
4.11
4.10
4.08
4.07
2.07 acetone-d6
1.48
1.48
1.36
1.35
1.34
1.34
0.96
0.19
0.18
1.00
1.09
1.96
0.80
0.94
1.04
3.01
1.02
3.28
8.58
3.04
8.74
2.83
2.74
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

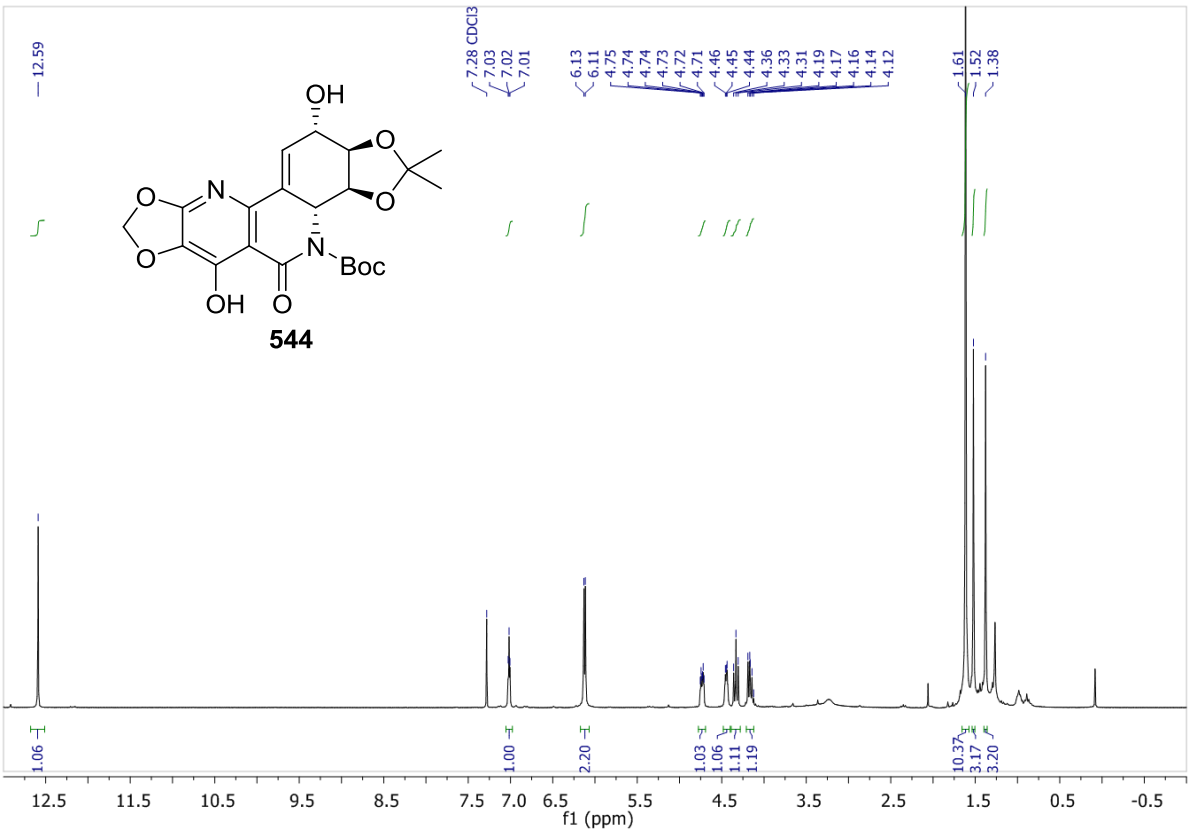

205.25 acetone-d6
165.00
164.76
159.21
159.14
151.48
146.28
145.86
130.53
130.44
127.05
126.87
125.70
124.59
123.97
123.93
108.09
108.05
101.34
84.04
83.96
79.84
79.76
75.32
71.31
71.28
59.69
59.65
27.25
27.22
26.90
25.35
24.93
17.82
-5.09
-5.54
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

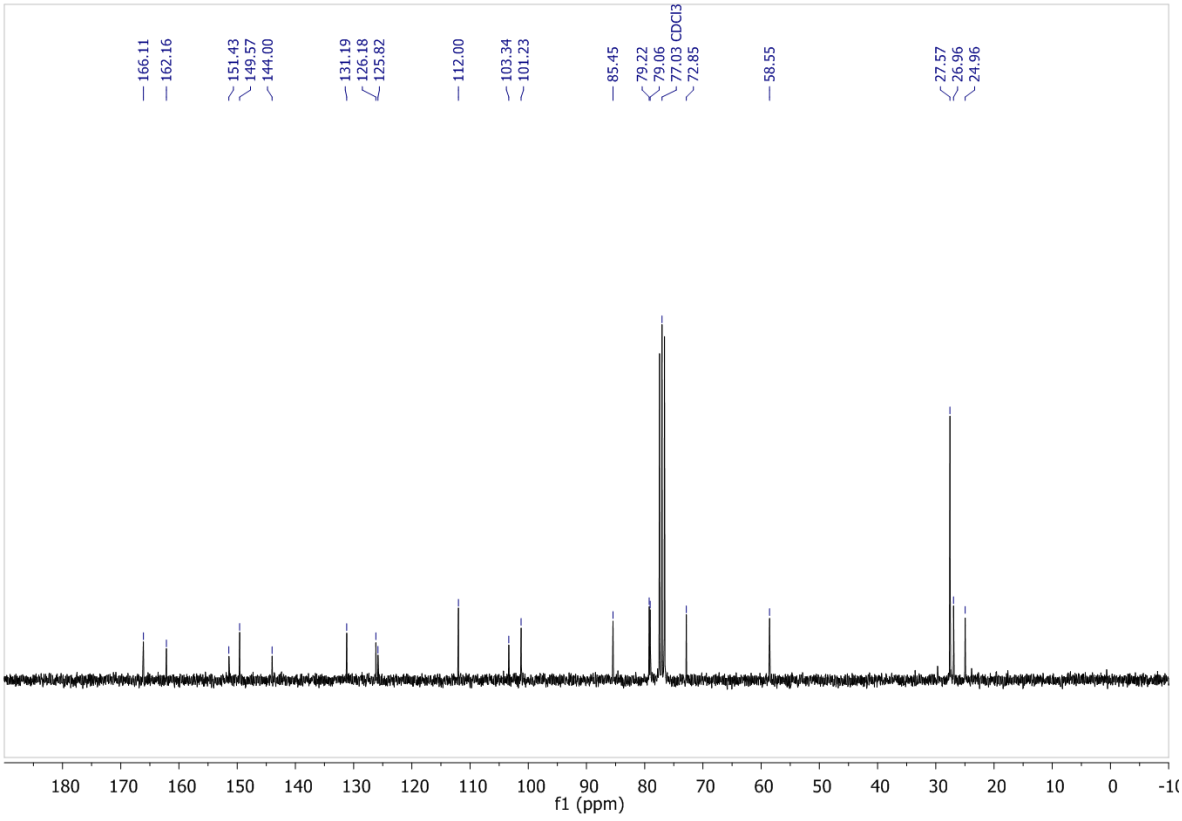

OTBS
O
O
N
O
O
N
Boc
OMe O
542
6.99
6.98
6.98
6.06
6.05
6.05
4.72
4.72
4.71
4.71
4.70
4.70
4.39
4.39
4.39
4.38
4.38
4.21
4.15
4.14
4.13
4.06
4.05
4.05
4.04
1.60
1.48
1.32
0.95
0.15
1.00
2.07
1.04
1.05
3.15
1.17
1.06
9.69
3.27
3.10
9.70
6.40
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

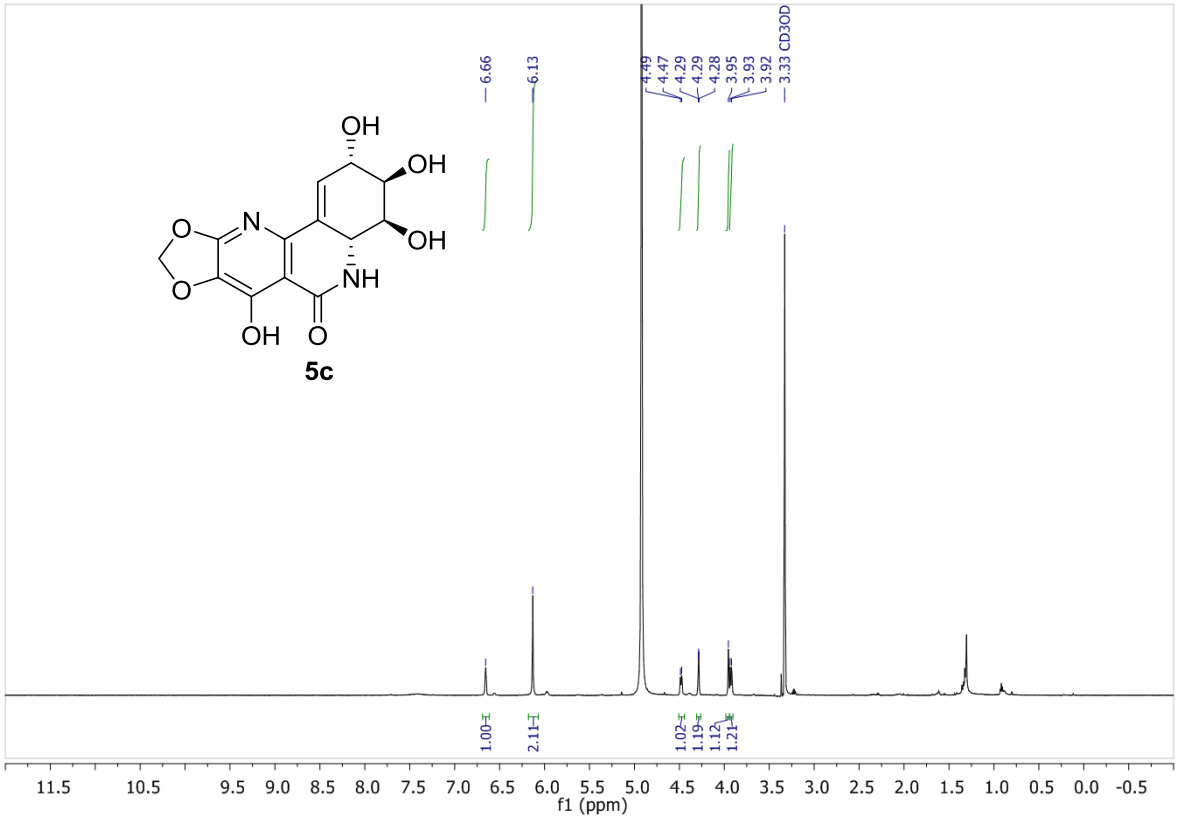

161.69
160.49
153.89
149.69
145.66
133.59
127.26
126.63
111.37
109.02
100.02
84.05
79.76
79.65
77.03 CDCl3
73.80
60.47
57.65
27.65
27.02
25.86
25.12
18.20
-4.52
-4.97
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


OTBS
O
O
N
O
O
N
Boc
OH
O
543
12.70
7.28 CDCl3
7.02
7.02
7.01
6.13
6.12
4.75
4.75
4.74
4.74
4.73
4.73
4.40
4.39
4.39
4.38
4.38
4.21
4.19
4.18
4.10
4.09
4.08
4.07
1.62
1.50
1.34
0.96
0.17
0.99
0.96
2.07
1.00
1.03
1.04
1.06
9.31
3.22
3.23
9.35
6.17
13.5
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
f1 (ppm)


166.27
162.19
151.76
149.75
144.38
133.58
125.61
111.57
103.46
101.06
85.30
79.36
77.03 CDCl3
73.60
58.39
27.60
26.98
25.83
25.07
18.18
-4.54
-4.98
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


12.59
7.28 CDCl3
7.03
7.02
7.01
6.13
6.11
4.75
4.74
4.74
4.73
4.72
4.71
4.46
4.45
4.44
4.36
4.33
4.31
4.19
4.17
4.16
4.14
4.12
1.61
1.52
1.38
OH
O
N
O
O
O
N
Boc
OH
O
544
1.06
1.00
2.20
1.03
1.06
1.11
1.19
10.37
3.17
3.20
12.5
11.5
10.5
9.5
8.5
7.5
7.0
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
f1 (ppm)


166.11
162.16
151.43
149.57
144.00
131.19
126.18
125.82
112.00
103.34
101.23
85.45
79.22
79.06
77.03 CDCl3
72.85
58.55
27.57
26.96
24.96
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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OH
N
O
O
NH
OH
O
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6.13
4.49
4.47
4.29
4.29
4.28
3.95
3.93
3.92
3.33 CD3OD
1.00
2.11
1.02
1.19
1.12
1.21
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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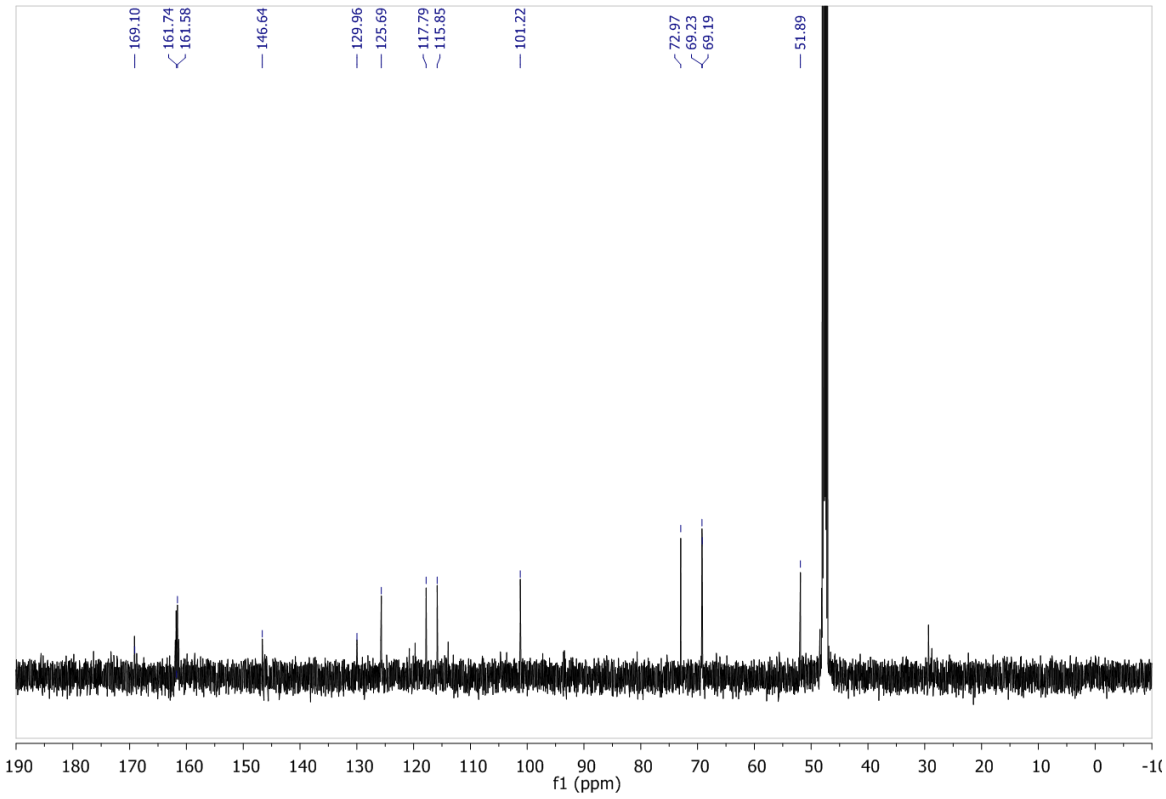
-0.5
f1 (ppm)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72.97
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170
160
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140
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110
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80
70
60
50
40
30
20
10
0
f1 (ppm)

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8. Vita

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